Department of Pharmacognosy
University of Szeged

Isolation and Structural Characterization of New Jatrophane Diterpene Polyesters from *Euphorbia serrulata* and *Euphorbia mongolica*

Ph.D. Thesis

Dóra Rédei

Szeged, Hungary
2004

List of publications related to the thesis

I Hohmann J, Rédei D, Evanics F, Kálmán A, Argay G, Bartók T: Serrulatin A and B, new diterpene polyesters from *Euphorbia serrulata*, *Tetrahedron* 2000; **56**: 3619-3623

II Mucsi I, Molnár J, Hohmann J, Rédei D: Cytotoxicities and anti-herpes simplex virus activities of diterpenes isolated from *Euphorbia* species, *Planta Med.* 2001; **67**: 672-674

III Hohmann J, Molnár J, Rédei D, Evanics F, Forgo P, Kálmán A, Argay G, Szabó P: Discovery and biological evaluation of a new family of potent modulators of multidrug resistance: Reversal of multidrug resistance of mouse lymphoma cells by new natural jatrophane diterpenoids isolated from *Euphorbia* species, *J. Med. Chem.* 2002; **45**: 2425-2431

IV Rédei D, Hohmann J, Evanics F, Forgo P, Szabó P, Máthé I: Isolation and structural characterization of new, highly functionalized diterpenes from *Euphorbia serrulata*, *Helv. Chim. Acta* 2003; **86**: 280-289

V Hohmann J, Rédei D, Forgo P, Molnár J, Dombi G, Zorig T: Jatrophane diterpenoids from *Euphorbia mongolica* as modulators of the multidrug resistance of L5128 mouse lymphoma cells, *J. Nat. Prod.* 2003; **66**: 976-979

Contents

Abbreviations and symbols

1D	one-dimensional
2D	two-dimensional
API-ES	atmospheric pressure ionization electrospray
COSY	correlated spectroscopy
crys	crystallization
δ	chemical shift
ESIMS	electrospray ionization mass spectroscopy
FAB	fast atom bombardment
fr	fraction
HMBC	heteronuclear multiple-bond correlation
HMQC	heteronuclear multiple-quantum coherence
HPLC	high-performance liquid chromatography
HRESIMS	high-resolution electrospray ionization mass spectroscopy
HRFABMS	high-resolution fast atom bombardment mass spectroscopy
HR-MS	high-resolution mass spectroscopy
HSQC	heteronuclear single quantum correlation
IR	infrared
JMOD	*J*-modulated spin-echo experiment
MDR	multidrug resistance
NOE	nuclear *Overhauser* effect
NOESY	nuclear *Overhauser* effect spectroscopy
NMR	nuclear magnetic resonance
NP	normal-phase
OCC	open-column chromatography
PLC	preparative-layer chromatography
RP	reversed-phase
TLC	thin-layer chromatography
TOCSY	total correlated spectroscopy
UV	ultraviolet
VLC	vacuum-liquid chromatography

"O, mickle is the powerful grace that lies
In herbs, plants, stones, and their true qualities..."
"Within the infant rind of this small flower
Poison hath residence, and medicine power..."

Shakespeare, "Romeo and Juliet" II,3

1. Introduction

The Euphorbiaceae, a family of about 8000 species in 300 genera, is one of the largest and most unwieldy families of the angiosperms, and because of the range of morphological variation it may be polyphyletic in origin.[1,2] Species of the spurge family produce the well-known and widely-used castor oil *(Ricinus communis)*, rubber (Havea, Manihot and Sapium species), manihot or cassava starch (*Manihot esculentus*), the dye and taenicide kamala (*Mallotus philippinensis*), croton oil (*Croton tiglium*), cascarilla bark (*Cascarilla eleuteria*) and the resinous 'euphorbium' (*Euphorbia resinifera*).[3,4] Many members of this family are used as drugs in folk medicine for the treatment of various diseases, and more than 150 of them against cancer.[5]

The phytochemical investigation of several Euphorbiaceae species started at the end of the 19th century with the aim of studying which of their constituents are responsible for the long-known irritant, pro-inflammatory and purgative activity. In 1935, BÖHM obtained from croton oil a crystalline, labile compound which he called 'phorbol' because of its alcohol nature. After systematic fractionation, employing mild and efficient methods of separation combined with careful follow-up of the physical and biological properties of all fractions, in the 1960s HECKER *et al.* elucidated the chemical structures of phorbol, a toxic diterpene of tigliane type, and its esters as the irritant and tumour-promoting constituents of croton oil.[6] Thereafter, many tigliane, ingenane and daphnane diterpenes with pro-inflammatory and cocarcinogenic effects were isolated from Euphorbiaceae and Thymelaeaceae species.

In 1937, DUBLINSKAJA reported the isolation of the first macrocyclic diterpene, 'euphorbiasteroid'. This compound did not exhibit pronounced toxicological action. Accordingly, little interest was shown in research into this type of diterpenes until 1970.[7] At that time, KUPCHAN isolated from *Jatropha gossypiifolia* the compound jatrophon, which displays activity against mouse P-388 lymphocytic leukaemia *in vivo*. Many macrocyclic diterpene esters with great structural variety and noteworthy biological activities were

subsequently isolated from Euphorbiaceae species.[8] The isolation and structure elucidation of macrocyclic diterpenes continue to pose new challenges and questions for researchers. The specific chemical structures and the newly-discovered biological activities of these compounds have aroused the interest of chemists, botanists and pharmacologists.[9,10,11,12,13]

In 1995, HOHMANN *et al.* (Department of Pharmacognosy, University of Szeged) initiated a research programme involving investigation of the secondary metabolites of plants of the *Euphorbia* species. In the course of these studies, a screening method for the diterpene content has been developed, and many diterpene esters of different skeletal types have been isolated.[14,15,16,17,18] One part of this programme related to the phytochemical investigation of *Euphorbia serrulata* Thuill. and *Euphorbia mongolica* Prokh.

1.1. Diterpenoids of the plant family Euphorbiaceae

Plants of the Euphorbiaceae are known to contain a large number of biologically active diterpenes with various chemical structures. These diterpenes can be classified into two groups: 'lower terpenes' derived from a tetraprenyl pyrophosphate precursor through a 'head-to-tail' cyclization, and non-specific 'higher terpenes', whose skeletons are formed by the classical 'concertina-like' cyclization typical of many diterpenoids, triterpenoids and steroids. The functionalization of diterpenes presumably proceeds after cyclization. The distribution of 'lower diterpenes' is limited in the Euphorbiaceae and Thymeleaceae, while 'higher diterpenes' occur in many plant families. Unfortunately, the 'lower diterpenoids' under discussion here are neither easily detected nor easily isolated; and not one species in the whole of the family Euphorbiaceae has yet been subjected to an exhaustive biosynthetic study.[19,20]

The hypothetical biosynthesis starts with the condensation of tetraprenyl pyrophosphate, initiated by loss of the diphosphate group (Figure 1). The cembrene cation produced is a very reactive intermediate with a 14-membered ring.[21] It is stabilized through the formation of cembranoids.[22] Such compounds have been isolated from various organisms. Besides phytocembranoids[23,24], numerous cytotoxic[25], anti-tumour[26] and HIV-1-inhibitory[27] compounds with many oxygen functions are known from marine invertebrates. The casbanes formed from the cembrene cation via loss of a proton and cyclization of the isopropane group have been considered to be precursors of a number of macrocyclic diterpenes.[7] Casbanes with

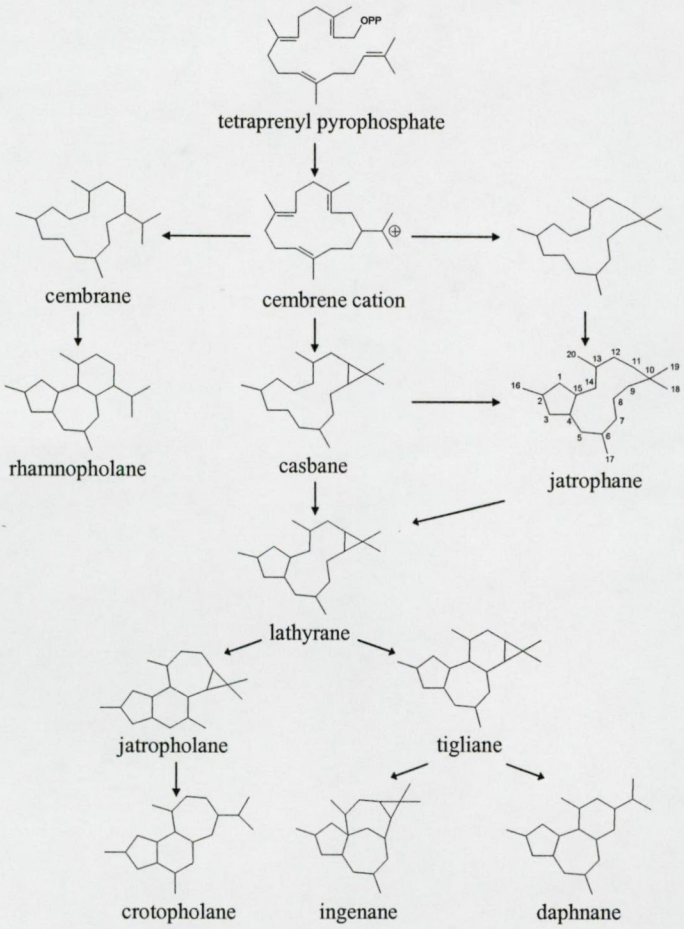


Figure 1. Hypothetical biogenic interconversions of diterpenes in the Euphorbiaceae

a *trans* or *cis* disubstituted cyclopropane ring occur in the species of *Euphorbia*[28,29] and other Euphorbiaceae genera[30,31,32,33]. The first known macrocyclic *Euphorbia* diterpene, 'euphorbiasteroid', was obtained in 1937 from the seed oil of *E. lathyris*.[34] Its structure, with a tricyclic lathyrane skeleton, was elucidated in 1970.[35,36] This type can be formed from casbane by further intramolecular cyclization between C-4 and C-15[7], or from jatrophane by cyclization between C-9 and C-11[37].* Lathyrane-type compounds do not possess the pro-inflammatory activity of phorbol esters.[38,39] Ingols, epoxy derivatives of lathyranes, have given rise to considerable interest as cytotoxic[40,41], vasoactive[42,43] and PGE_2-inhibitory[44] agents.

---

* The skeletons of lathyranes and casbanes differ from that of jatrophanes in the additional intramolecular cyclizations. Thus, the jatrophane numbering has been used here (Figure 1).

Tigliane, ingenane, daphnane, jatropholane, crotopholane and rhamnopholane are further common types of 'lower diterpenes' with tri- and tetracyclic skeletons.[20,45] The first discovered compounds containing a tigliane, ingenane or daphnane framework became famous for their cocarcinogenic and skin-irritant activities.[7] They proved most useful in mechanistic studies of the carcinogenic process. The most exhaustively studied tiglianes are phorbols esterified at C-12 and C-13, which can activate protein kinase C (PKC); they may therefore disturb the enzyme-regulated cellular activity. 12-*O*-Tetradecanoylphorbol 13-acetate (TPA) became a classical activator of PKC in many studies relating to signal transduction.[46] Some of the later isolated tiglianes exert anti-HIV-1 activity,[47,48] a cytoprotective effect in lymphocytic cells infected with HIV-1[49] or selective cytotoxicity for human kidney carcinoma[50]. Ingenane esters are widespread in the Euphorbiaceae. Specific members of this type are milliamines because of their peptidyl ester structures[51,52]. Ingenanes isolated from the Asian plant *E. kansui* have noteworthy biological activities[53,54,55]. Daphnane derivatives have been isolated from genera of the Thymelaeaceae (*Daphnae*,[56,57] *Thymelaea*,[58] *Gnidia*,[59] *Synaptolepis*[60] and *Wikstroemia*[61]) and Euphorbiaceae (*Euphorbia*,[62] *Excoecaria*,[63] *Neoboutonia*[64] and *Maprounea*[65]) families. Resiniferatoxin and related compounds containing a homovanillic acid ester moiety are ultrapotent capsaicin analogues; they may therefore be of clinical relevance.[66] A series of new diterpene polyesters with uncommon skeletons have recently been described, such as myrsinanes,[67,68,69] cyclomyrsinanes,[70,71] segetanes[72] and paralianes[73,74] derived from macrocyclic compounds via further intramolecular C-C bond formation.

Besides their pharmacological relevance, the 'lower diterpenoids' are the most important taxonomic markers in the Euphorbiaceae family in consequence of their extraordinary structural diversity and limited distribution in the plant kingdom.[12]

Among the diterpene constituents of the Euphorbiaceae family, non-specific 'higher diterpenes' may be mentioned, such as the bicyclic labdane[75,76] and clerodane[77,78], the tricyclic abietane[79,80] and the tetracyclic bayerane,[81,82] kaurane[81] and atisane[83,84] types.

1.2. Jatrophane diterpenes

The jatrophane type may be formed in nature from tetraprenyl pyrophosphate through an intermediate with a 15-membered macrocycle or through a casbene precursor by a further cyclization step and opening of its cyclopropane ring (Figure 1).[7,20] Jatrophon, the first compound isolated with a jatrophane skeleton, demonstrated significant activity against P-388 lymphocytic leukaemia.[8] Since the 1970s, more than 170 jatrophanes from natural sources have been identified. Their great structural variability stems from the number and positions of the double bonds, the nature and number of the oxygen functions, the esterification pattern and the configuration of the diterpene core.

Jatrophanes have a bicyclo[10.3.0]pentadecane ring system, most frequently featuring a C-11/C-12*E* double bond and an exocyclic 6(17) methylene group. There is an additional C-5/C-6 double bond in compounds isolated from *E. maddenii*,[85] *E. pubescens*,[86] *E. characias*[87] and *E. helioscopia*[88], and they therefore have a methyl group on C-6. The unusual euphornin E contains not only a disubstituted C-11/C-12 and a trisubstituted C-5/C-6 double bond, but also a fully substituted one at C-15/C-16 in the macrocycle.[89] Some characiol derivatives are noteworthy because of their C-5/C-6*E* and C-12/C-13*E* double bonds.[87]

Besides hydroxy groups (present in 61% of jatrophanes), the parent alcohols can contain keto (85%), epoxy (12%) and ether (6%) groups as oxygen functions.* Hydroxy groups can usually be found on C-3, C-5, C-7, C-9 and C-15, but also on C-2,[90,91] C-8[92] and C-14[89], and rarely on C-1,[93] C-6[94,95] and C-13[96,97]. Keto groups are usually located at C-9 or/and C-14, and occasionally at C-7[89] or C-12[98]. Jatrophanes with an 11,12-epoxy group have been isolated from *E. salicifolia*,[94,99] *E. esula*[100] and *E. kansui*[93], and others with a 5,6-epoxy group from *E. characias*[87] and *E. helioscopia*[101]. Kansuinin A[93] and D,[102] esulatin C[14] and euphohelin A-E[101] have tricyclic structures because of the tetrahydrofuran ring formed by the ethereal oxygen bridge between C-11 and C-14 or C-12 and C-15. Terracinolides[97,103,104], isoterracinolides[96] and salicinolide[94] are C_{22} lactones bearing an additional two-carbon segment linked to C-17. One of these two carbon atoms is oxidized at the carboxylic acid level and is cyclized with a proximate hydroxy group on C-5, C-3 or C-9 to give a 6- or

* These data are based on 44 publications describing the isolation and structure elucidation of 171 new jatrophane diterpenes (in the period 1971-2003).

8-membered lactone ring. Euphosalicin, an 11(10→18)*abeo*-jatrophane derived by incorporation of a geminal methyl group in the ring system[99], and the 1(15→14)*abeo*-jatrophanes from *E. dendroides*[97] and *E. terracina*[107], are uncial rearranged compounds.

Plants produce polyacylated derivatives of jatrophane alcohols. The number of ester moieties ranges between 2[87,89] and 8[95]. The acyl residues are of various structural types, with most frequently acetate (in 94% of jatrophanes), benzoate (54%), isobutyrate (39%), 2-methyl butyrate (10%) and nicotinate (9%), and more rarely propionate (4%), tigliate (2%), cinnamate (2%), butyrate (2%) and angelate (1%) moieties.* The most heterogeneously esterified molecules have 4 different acyl residues.[18,105] There are a few compounds with homogeneous ester groups.[15,90,106,107]

Depending on their substitution pattern, jatrophanes may have 5–10 chiral centres. The ring junction of the 2 carbocycles of the compounds known to date always proves *trans*, and the position of the substituent at C-5, C-8 and C-9 is α and that at C-3, C-6 and C-7 is β relative to the angular H-4, which is assumed to be α-oriented. The reason why these diterpenes do not form a stereochemically homogenous series is that the configuration of the remaining carbons (C-1,[95,108] C-2,[89,109,110] C-13[89,105] and C-14[89,96]) is variable.

The two-dimensional (2D) representation of the jatrophane skeleton offers an opportunity for a number of drawing modes and orientation. The absolute configuration of natural jatrophanes is such that, if the molecule is oriented with the 5-membered ring to the left of the observer, H-4 points away. An alternative representation, with the 5-membered ring to the right, has also been employed. In this case, H-4 points towards the observer. APPENDINO *et al.* extended certain conventions that had earlier been proposed for medium-sized sesquiterpenoids so that they also related to medium-sized diterpenoids. The perimeter of the ring may be drawn so that it clearly portrays the *E-Z* character of the double bond, but bearing in mind the need to avoid re-entrant angles at tetrahedral carbon atoms.[111,112,113]

Structure-activity relationship studies have highlighted the importance of molecular conformation analyses. Notwithstanding an identical configuration at the stereogenic centres, their flexibility allows the jatrophanes to adopt different conformations, depending mainly on their substitution pattern. The 6(17),11-dienes are best described in terms of the orientation of

---

* These data are based on 44 publications describing the isolation and structure elucidation of 171 new jatrophane diterpenes (in the period 1971-2003).

the 6,17-exomethylene group, which is perpendicular ("endo") or parallel ("exo") to the plane of the 12-membered ring.[105]

1.3. Botany of the Euphorbiaceae

The Euphorbiaceae comprise one of the largest and most diversified families of angiosperms. There is great diversity in growth form, from tall rain forest trees to lianas, shrubs, perennial and annual herbs, geophytes, succulents, and floating aquatics; only the epiphytic habit is lacking among the major 'niches' of vegetative adaptation. Although all taxa have unisexual flowers, pollination is achieved by means of a wide variety of agents, including the wind, insects, birds, bats and non-flying mammals.[1]

1.3.1. Classification of *E. serrulata* and *E. mongolica*

Species:	*Euphorbia serrulata* Thuill.	*Euphorbia mongolica* Prokh.[114]
Subsectio:	Galarrhaei (Boiss.)	Rupestres (Prokh.)
Sectio:	Tithymalus (Boiss.)[7]	Holophyllum (Prokh.)[115]
Genus:	Euphorbia	
Tribe:	Euphorbieae[2]	
Subfamily:	Euphorbioideae	
Family:	Euphorbiaceae	
Order:	Euphorbiales[116]	

Euphorbiaceae is the 'core' family of the order Euphorbiales. The question of the relationship of the Euphorbiales to other suprafamilial groups of dycotyledons remains unresolved. In several systematic classifications, the order Euphorbiales is associated with various other orders in the superorder Euphorbianae or Malvanae. Most authors include the Euphorbiales in subclass Rosidae, whereas others do so in the Dilleniidae. CRONQUIST suggested placing the order in a superorder that straddles the arbitrary boundary between the Rosidae and the Dilleniidae.[1,116,117,118]

1.3.2. Botanical description of *E. serrulata* and *E. mongolica*

The upright spurge, *Euphorbia serrulata* Thuill. (nom. illegit. *E. stricta* L., *E. platyphyllos* var. *stricta* Neilr., *Tithymalus strictus* Klotsch et Garcke[117]), is a monoecious plant with latex; it is always glabrous, branching and slender annual herb, with a height of 60–80 cm, and has an umbel with 4–5 rays. Stipules are absent; its leaves are alternate, simple, obovate- to oblong-lanceolate, serrulate, acute, and deeply cordate at the base. The raylet-leaves become narrower downwards and gradually pass into the ray-leaves. The flowers are apetalous, without sepals, in small groups surrounded by an involucre, with 4–5 transversely ovate, entire glands at the top, the whole forming a cyathium. The male flowers of a single stamen are joined to the pedicel. The female flowers are solitary, pedicellate, surrounded by several male flowers. The ovary is superior, 3-locular, with 3 styles. The ovules are solitary in each loculus. The 'inflorescence' of the cyathia is umbellate. Bracts are present between the male flowers. The red-brown, indurate and dehiscent capsule measures 2.5 mm or less; it is deeply sulcate, and covered with cylindrical tubercles which are longer than wide. The seeds are smooth and reddish. The plant flowers in Hungary from June to August. It occurs in river floodplain woods and gallery forests from Central and South-east Europe to the Caspian Sea; it is adventive in North America.[119,120]

Euphorbia mongolica Prokh. is a grey-green, glabrous, pruinose, monoecious perennial herb with a height of 10–45 cm. White milky latex is obtained by incision of the plant. The roots are black-brown, 2–3 cm thick, juicy and branching. On the bottom of the erect shoot are withered remains of the previous year's stems. The plant has numerous flowering and non-flowering shoots. The red-brown lower leaves are 10 mm wide, membranous and caducous. The greyish pale-green middle leaves are alternate, simple, entire, ovate-lanceolate, obtuse, narrowed at the base, 2.5–4.5 cm long and 1–1.5 cm wide. The 'inflorescence' formed by the cyathia is umbellate with 5 rays. The ray-leaves are ovate-triangular, rounded or cordate at the base, and gradually pass into the triangular raylet-leaves. The lower parts of the reniform light-brown glands are pruinose. The ovate-globular and shallowly sulcate capsules, with a diameter of 6-7 mm, are pruinose and glabrous when unripe, and covered with tubercles when ripe. The dark-brown seeds are 4 mm long and compressed-ovoid. It occurs on stony slopes, rocky places and stone-slides in Mongolia and East-Siberia.[115,121]

1.4. Chemistry and folk medicinal use of *E. serrulata* and *E. mongolica*

KINGHORN & EVANS tested 60 species of the *Euphorbia* genus for dermal pro-inflammatory effects, using the mouse ear test. The extract of *E. serrulata* demonstrated low irritant activity as compared with other extracts.[122] A comparative phytochemical study of the tigliane and ingenane diterpenes of 57 *Euphorbia* species was described. After alkaline hydrolysis and acetylation of the diterpene-containing fractions of the acetone extracts, it was found that the latex of *E. serrulata* contains ingenol esters, which are the most common type in the genus *Euphorbia*, and 5-deoxyingenol derivatives.[123] POHL *et al.* investigated the flavonoids of 4 *Euphorbia* species native in Germany. They isolated quercetin-3-β-galactoside and quercetin-3-β-arabinoside from methanol extract of the dried flowering aerial parts of *E. serrulata*. The presence of quercetin glucosides in this species could not be proved.[124] Neither any folk medicinal use nor any pharmacological investigation of the plant has yet been published.

E. mongolica is traditionally used in Mongolia to treat inflammation, warts and tumours.[125] In contrast with its apparent medicinal benefit, no data on the chemical composition of the species have previously been reported.

2. Aims of the study

The widespread genus *Euphorbia* is the source of a large number of biologically active isoprenoids. Besides the well-known skin-irritant and tumour-promoting tigliane, ingenane and daphnane diterpenes, considerable attention has been paid to the macrocyclic diterpenes, because of their high chemical diversity and therapeutically relevant bioactivity. In 1995, HOHMANN *et al.* (Department of Pharmacognosy, University of Szeged) initiated a research programme with the aim of the investigation of the secondary metabolites of plants of the *Euphorbia* species. The aims of the present work, as part of that programme, were the isolation and structural characterization of new diterpene polyesters which may have potential pharmacological effects.

In order to achieve these aims the main tasks were:

- The screening of *E. serrulata* and *E. mongolica* (2 species without any previous detailed chemical analysis) for diterpene content.
- The extraction of the plant material.
- The isolation and purification of diterpene esters by a combination of various chromatographic methods [open column chromatography (OCC), vacuum-liquid chromatography (VLC), preparative-layer chromatography (PLC) and high-performance liquid chromatography (HPLC)].
- The characterization and structure determination of the isolated compounds by different spectroscopic techniques [nuclear magnetic resonance (NMR) spectroscopy, high-resolution mass spectroscopy (HR-MS), ultraviolet (UV) and infrared (IR) spectroscopy)].
- Evaluation of the pharmacological potential and chemotaxonomical relevance of the isolated diterpenes.

3. Materials and methods

3.1. Plant material

E. serrulata

The whole plants were collected in June 1998 from the hill-country near Iklódbördöce, Country Zala, Hungary, and identified by Tamás Rédei*. The 2410 g of fresh plant material was frozen and stored at –15 °C until preparation.

E. mongolica

The plant material was obtained by Tomur Zorig†. Aerial parts of *E. mongolica* were gathered in Govi Gurvan-Sajhan, Omnigovi, Mongolia, in June 2001, and identified by S. Sanzhir†. The plant material was air-dried at room temperature. The 171 g of dried raw material was stored at room temperature until preparation.

3.2. Screening of plant material for diterpene content

10 g of frozen and crushed (*E. serrulata*) or 5 g of dried and powdered (*E. mongolica*) plant material was percolated with 100 ml of methanol at room temperature. After concentration, 10 ml of water was added to the extract (10 ml) and it was subjected to solvent partitioning with 3 x 20 ml of dichloromethane. The dichloromethane-soluble phase was subjected to polyamide (0.5 g) OCC using a gradient system of methanol–water [6:4, 8:2 and 1:0 (150 ml each)] as eluent. The fractions were concentrated and monitored by thin-layer chromatography (TLC) using mobile phases A and D (described in section **3.3.2**).

3.3. Extraction and isolation of diterpenes

3.3.1. Extraction

E. serrulata: The frozen plant material was crushed with a Waring CB-6 commercial blender (model 33BL13). The raw material was percolated with 19 l of methanol at room temperature in a glass percolator (diameter 15 cm, height 35 cm). Solvent partitioning was carried out with 6 x 300 ml of *n*-hexane.

---

* Institute of Ecology and Botany of the Hungarian Academy of Sciences, Vácrátót, Hungary

† Institute of Medicinal Research, Section of Pharmacy, Ulanbaatar, Mongolia

E. mongolica: The dried plant material was powdered with a Bosch MKM 6000 grinder. The raw material was percolated with 6700 ml of methanol at room temperature in a glass percolator (diameter 10 cm, height 25 cm). Before the liquid-liquid extraction with 5 x 400 ml of dichloromethane, 250 ml of water was added to the concentrated extract.

3.3.2. Isolation and purification of compounds

Open column chromatography: OCC was performed on polyamide (ICN) for column chromatography (72.0 g for the *E. serrulata* extract and 48.0 g for the *E. mongolica* extract). The dissolved extract was added to one-third of the polyamide, and the solvent (methanol) was totally eliminated. The resulting powdery material was then placed at the top of a chromatographic column filled with the remainder of the stationary phase. Mixtures of methanol–water [3:2 and 4:1 (1000 ml each)] were used as mobile phase for both plant extracts.

Vacuum-liquid chromatography: For VLC, TLC-silica gel 60 G (mean particle size 15 μm) (Merck 11677) was used. The dissolved extract was added to one-tenth of the silica gel. After drying, the powdery material was placed on the remainder of the stationary phase in a filter funnel. The VLC column was developed under gentle vacuum, provided by a water pump.

Column 1 eluents: petroleum ether (40-60 °C)–ethyl acetate [49:1, 9:1, 4:1, 7:3 and 1:1 (500 ml each)]; volume of collected fractions: 50 ml; sorbent: 60 g

Column 2 eluents: benzene–chloroform–diethyl ether [10:5:1, 10:6:2, 10:7:3, 10:9:4 and 2:2:1 (100 ml each)]; volume of collected fractions: 10 ml; sorbent: 12 g

Column 3 eluents: benzene–chloroform–diethyl ether [10:5:1, 10:6:2, 10:7:3, 10:9:4 and 2:2:1 (100 ml each)]; volume of collected fractions: 10 ml; sorbent: 10 g

Column 4 eluents: cyclohexane–ethyl acetate–ethanol [70:10:0, 70:20:0, 70:20:1, 70:20:2 and 70:30:3 (50 ml each)]; volume of collected fractions: 50 ml; sorbent: 95 g

Column 5 eluents: dichloromethane–acetone [100:0, 99:1, 98:2, 97:3, 95:5 and 90:10 (50 ml, 4 x 75 ml and 50 ml)]; volume of collected fractions: fractions 1–5 and 66–71: 10 ml, fractions 6–65: 5 ml; sorbent: 11.75 g

Layer chromatography: PLC was performed on silica gel 60 plates (Merck 05715). Separation was monitored in UV light at 254 nm. Compounds were eluted from the scraped adsorbent with chloroform.

The obtained OCC and VLC fractions were monitored by TLC on silica gel 60 sheets (Merck 05554).

Visualization methods:

UV light: the TLC plate was exposed to UV light at 254 nm.

Conc. H_2SO_4: after spraying with conc. H_2SO_4, the TLC plate was heated at 110 °C for 5 min.

Mobile phases:

A: cyclohexane–ethyl acetate–ethanol (30:10:1)

B: chloroform–acetone (97:3)

C: chloroform–acetone (9:1)

D: chloroform–acetone (19:1)

Isocratic high-performance liquid chromatography: HPLC was carried out on a pre-packed Hibar RT (250 mm, 4 mm) LiChrospher Si 100 (5 μm) normal-phase (NP) column (Merck) and on a pre-packed Hibar RT (250 mm, 4 mm) LiChrospher RP-18 (5 μm) reversed-phase (RP) column (Merck), using a Waters Millipore instrument: Solvent Delivery System 6000A, Absorbance Detector 441, Data Module 730, Injector Rheodyne 7725I. The eluted compounds were detected at 254 nm.

NP methods:

Method 1 eluent: cyclohexane–ethyl acetate–ethanol (70:10:1); flow rate: 0.5 ml/min

Method 2 eluent: cyclohexane–ethyl acetate–ethanol (70:10:1); flow rate: 0.4 ml/min

Method 3 eluent: cyclohexane–ethyl acetate–ethanol (130:30:3); flow rate: 0.4 ml/min

RP methods:

Method 4 eluent: acetonitrile–water (4:1); flow rate: 0.5 ml/min

Method 5 eluent: acetonitrile–water (7:3); flow rate: 0.5 ml/min

Extracts and fractions were concentrated under vacuum with a Rotavapor RE (Büchi) rotary evaporation system, dipped in a water bath not warmer than 45 °C.

3.4. Characterization and structure determination of the isolated compounds

Mass spectroscopy: Electrospray ionization mass spectroscopy (ESIMS) measurements were carried out on a Hewlett-Packard 5989B MS Engine mass spectrometer equipped with an atmospheric pressure ionization electrospray (API-ES) interface (Hewlett-Packard 59987A).

Samples were introduced into the API-ES ion source by using a Harvard 22 syringe pump. High-resolution fast atom bombardment mass spectroscopy (HRFABMS) measurements were carried out on a VG ZAB2-SEQ high-resolution mass spectrometer. Fast atom bombardment (FAB) ionization with glycerol as matrix was used for measurements; the resolution setting was 10 000. High-resolution electrospray ionization mass spectroscopy (HRESIMS) measurements were carried out on a Perkin-Elmer Q-STAR Pulsar Q-TOF mass spectrometer equipped with an electrospray ion source.

Infrared and ultraviolet spectroscopy: IR spectra were obtained in KBr discs on a Perkin-Elmer Paragon 1000 PC FTIR instrument. UV spectra were obtained in methanol on a Shimadzu UV-2101 PC spectrometer.

Nuclear magnetic resonance spectroscopy: NMR spectra were recorded in $CDCl_3$ or C_6D_6 on a Bruker Avance DRX 500 spectrometer at 500 MHz (^1H) and 125 MHz (^{13}C). The signals of the deuterated solvents were taken as reference. Chemical shifts are given in ppm. 2D experiments were performed with standard Bruker software.

X-ray crystallographic study: A crystal was mounted on an Enraf-Nonius CAD4 diffractometer equipped with a graphite monochromator. Reflections were collected by using Cu Kα radiation and the ω-θ scan mode. The structure was solved by direct methods (Sheldrick, G.M. SHELXS-97 Program for Crystal Structure Solution, University of Göttingen) and refined by full-matrix least-squares (Sheldrick, G.M. SHELXL-97 Program for Crystal Structure Refinement, University of Göttingen).

Optical rotation: Optical rotations were determined in chloroform by using a Perkin-Elmer 341 polarimeter.

4. Results

4.1. Isolation of diterpenes

4.1.1. Screening of *E. serrulata* and *E. mongolica* for diterpene content

The extraction and sample preparation were carried out as described in section **3.2.** As concerns the screening results on *E. serrulata* and *E. mongolica*, the thin-layer chromatograms of the fractions eluted with 60% aqueous methanol yielded brown and blue spots of diterpenes with R_f values of 0.18–0.62 (mobile phase A, visualized by the conc. H_2SO_4 method). TLC monitoring of the fractions eluted with a 4:1 mixture of methanol–water revealed brown spots between Rf 0.67 and 0.95, suggesting that these fractions are rich in triterpenes and fats. The fractions obtained from the polyamide column with methanol contained much chlorophyll. This observation led to the conclusion that the extracts of *E. serrulata* and *E. mongolica* contain a series of different diterpene esters, which can be enriched in the 60% aqueous methanol fractions of the apolar extracts of the plants.

4.1.2. Isolation of diterpenes from *E. serrulata*

The extraction and isolation procedure, involving a combination of various chromatographic methods, is outlined in Figure 2. Since diterpene polyesters have been isolated from different organs of *Euphorbia* species, the whole plant of *E. serrulata* was collected. The *post mortem* enzymatic processes were stopped by freezing the fresh plants. The crushed plant material (2400 g) was percolated with methanol, an amphipolar solvent. The crude extract was concentrated in vacuum to 500 ml, and was then subjected to solvent partitioning to remove polar compounds. After exhaustive extraction with *n*-hexane, the organic phase was concentrated. The dark-green oily residue (16 g) was subjected to adsorption chromatography on an open polyamide column with methanol–water mixtures of decreasing polarity in order to remove the chlorophyll and triterpenes from the extract. The fraction eluted with methanol–water (4:1) contained triterpenes, fats and chlorophyll, and was not investigated further. The diterpene-containing fraction obtained with methanol–water (3:2) was dried in vacuum. The brown residue (1.50 g) was subjected to VLC on silica gel with a mean pore diameter of 60 Å. In this purification step, silica gel with a mean particle size of 15 μm was used to increase the selectivity. The hydrostatic pressure of the mobile phase was not high enough to overcome the

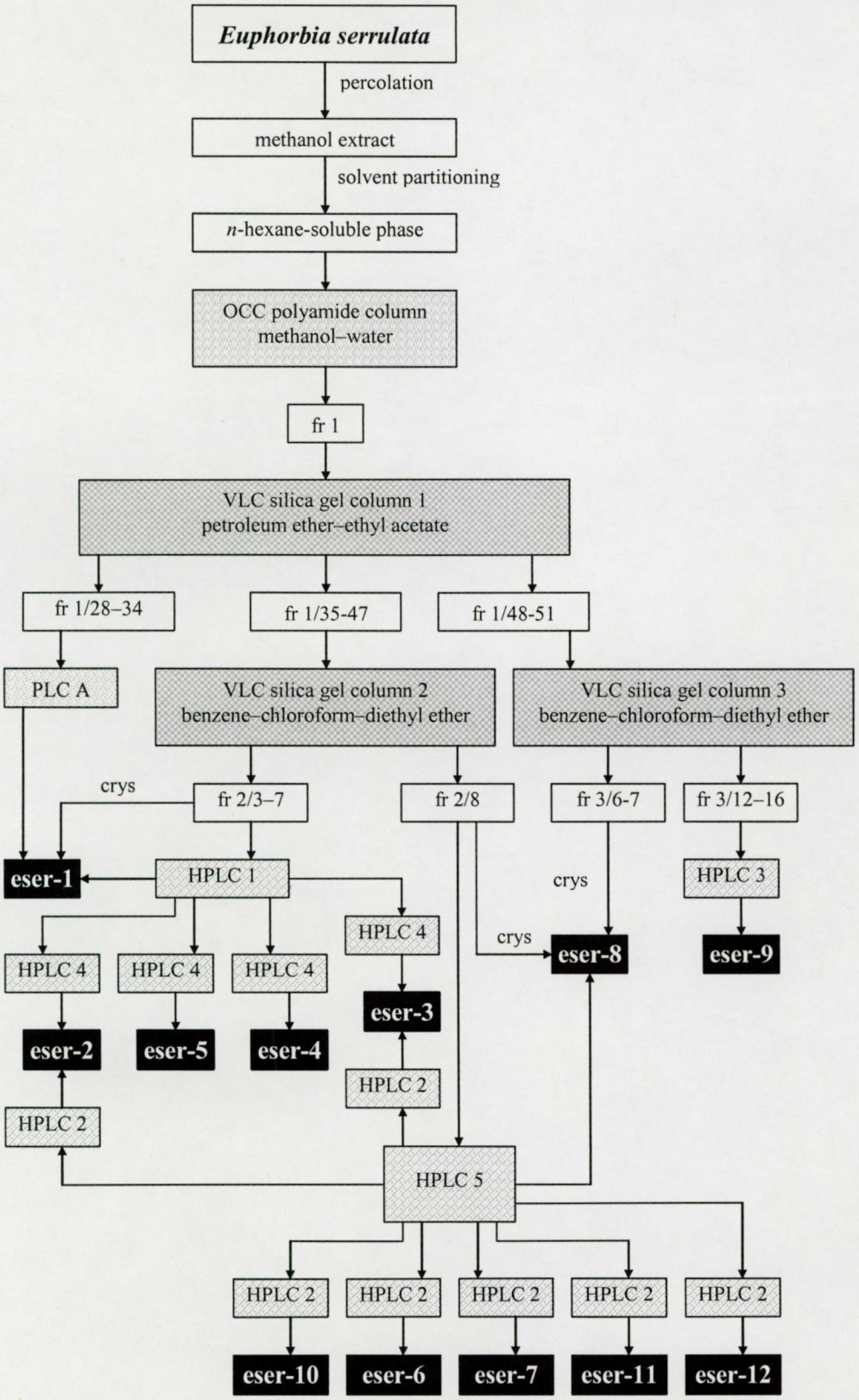


Figure 2. Isolation of diterpenes from *E. serrulata*

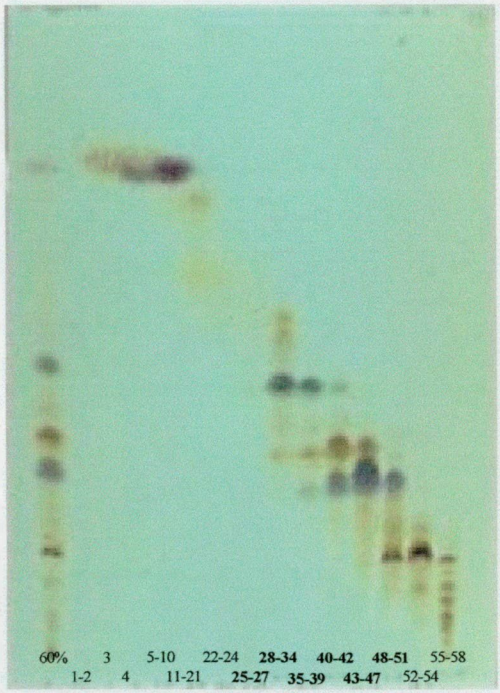


Figure 3. Thin-layer chromatogram of fractions eluted from VLC column 1 after the first combination step (mobile phase A, visualizing method: conc. H_2SO_4)

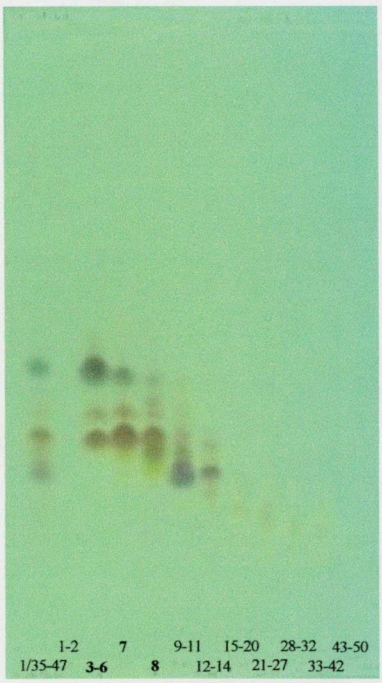


Figure 4. Thin-layer chromatogram of combined fractions eluted from VLC column 2 (mobile phase A, visualizing method: conc. H_2SO_4)

fluid resistance of the relatively small particles, which necessitated the application of a vacuum. The VLC separation on column 1 was carried out with a gradient system of petroleum ether–ethyl acetate mixtures of increasing polarity as eluent. The result of this fractionation was monitored by TLC, as illustrated in Figure 3. At least 7 spots of different diterpene esters were detected on the thin-layer chromatogram of fractions 1/28-34, 1/35-39, 1/40-42, 1/43-47 and 1/48-51.

From fraction 1/28-34, eser-1* (3.1 mg) was isolated by PLC on silica gel with the use of mobile phase A. For further purification, fractions 1/35-39, 1/40-42 and 1/43-47, eluted with petroleum ether–ethyl acetate (7:3), were combined. The dry residue (376.4 mg) of fraction 1/35-47 was subjected to repeated silica gel VLC (column 2). The fractions obtained were combined in 11 sub-fractions according to their composition, as indicated by TLC (Figure 4). In fractions 2/3-6, 2/7, 2/8 and 2/9-11 eluted with benzene–chloroform–diethyl ether (10:5:1 and 5:3:2), the main component of fraction 1/35-47 accumulated. The UV-light absorption of

* The compounds isolated in the present work from *E. serrulata* form the eser series (eser-1–12).

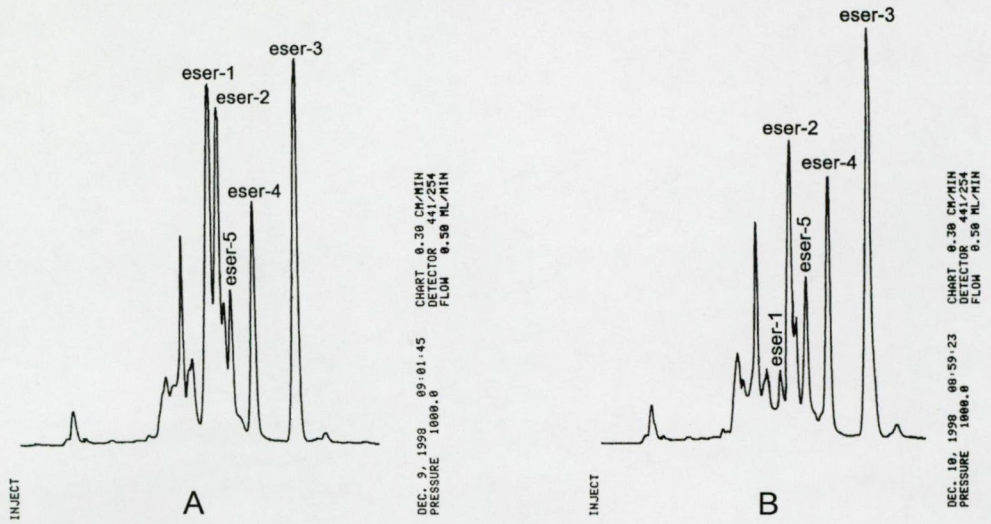


Figure 5. Separation of constituents of fraction 2/3-7 by NP HPLC using method 1; A: before crystallization of eser-1; B: after crystallization of eser-1

the TLC spots of the compounds allowed the final separation and purification by HPLC, with UV detection at 254 nm. Fraction 2/3-7 was fractionated by isocratic NP HPLC using method 1 (Figure 5). The compound observed at a retention time of 21.20 min crystallized from the solution and yielded colourless prisms of eser-1 (9.9 mg). The compounds at retention times of 22.15, 23.61, 26.08 and 30.80 min were separated and, after RP HPLC purification (using method 4), afforded eser-2 (5.9 mg), eser-5 (1.0 mg), eser-4 (10.6 mg) and eser-3 (38.8 mg), respectively. Fraction 2/8 obtained from the VLC column 2 with the first eluent was chromatographed by isocratic RP HPLC using method 5. The compound detected at a retention time of 22.68 min crystallized from the eluent and furnished colourless crystals of eser-8 (14.4 mg). The compounds eluted at retention times of 17.61, 25.75, 28.32, 34.25, 34.73 and 39.12 min were separated and, after NP HPLC purification using method 2, yielded eser-10 (2.3 mg), eser-6 (2.2 mg), eser-7 (5.9 mg), eser-11 (1.0 mg), eser-3 (9.3 mg) and eser-12 (1.0 mg), respectively. The dry residue (123.7 mg) of fraction 1/48-51 obtained with petroleum ether–ethyl acetate (1:1) from VLC column 1 was subjected to repeated VLC using column 3. A noteworthy amount (7.5 mg) of eser-8 was crystallized from fraction 3/6-7 eluted with benzene–chloroform–diethyl ether (10:5:1). Fraction 3/12-16 obtained with (10:6:2) was further fractionated by isocratic NP HPLC using method 3. The compound observed at a retention time of 20.75 min crystallized from the eluent and afforded white needles of eser-9 (5.2 mg).

4.1.2. Isolation of diterpenes from *E. mongolica*

The extraction and isolation procedure is depicted in Figure 6. The plant material was dried in Mongolia because of the long transport time to Hungary. The powdered raw material (166 g) was percolated with methanol at room temperature. After concentration under vacuum, the crude extract (350 ml) was diluted with water and subjected to solvent partitioning to yield a dichloromethane-soluble phase and a water-soluble phase. The concentrated dark-green, oily organic phase (12.59 g) was chromatographed on a polyamide column. The fraction obtained with a mixture of methanol-water (3:2) contained the diterpenes. The solvent of this fraction was removed by distillation under reduced pressure and the dry residue (2.37 g) was fractionated by VLC on column 4. Collected fractions were monitored by TLC, and combined in accordance with their composition. The thin-layer chromatograms of the fractions showed that the main diterpene constituents of the *E. mongolica* extract were present in fractions 4/22-26 and 4/32-38.

From fraction 4/22-26 obtained by VLC with mixtures of cyclohexane–ethyl acetate–ethanol (70:20:1), mf-1* (20.4 mg) was crystallized. Fraction 4/32-38 (0.277 g) eluted with a mixture of cyclohexane–ethyl acetate–ethanol (70:20:2) was subjected to silica gel VLC using column 5. The fractions obtained by this separation procedure were combined on the basis of TLC monitoring. From fraction 5/34–43 eluted with dichloromethane-acetone (98:2) and (97:3), mf-2 (13.9 mg) was obtained by crystallization. A small quantity of this compound (2.1 mg) was purified by PLC from the mother liquor with mobile phase B. Mf-3 (5.8 mg) crystallized from the eluent of the fraction 5/44-55 obtained with dichloromethane–acetone (97:3) and (95:5). The mother liquor of mf-3 was subjected to PLC. After development of the chromatogram with mobile phase C, a further amount of pure mf-3 (1.5 mg) was separated.

* The compounds that we isolated from *E. mongolica* comprise the mf series (mf-1–3).

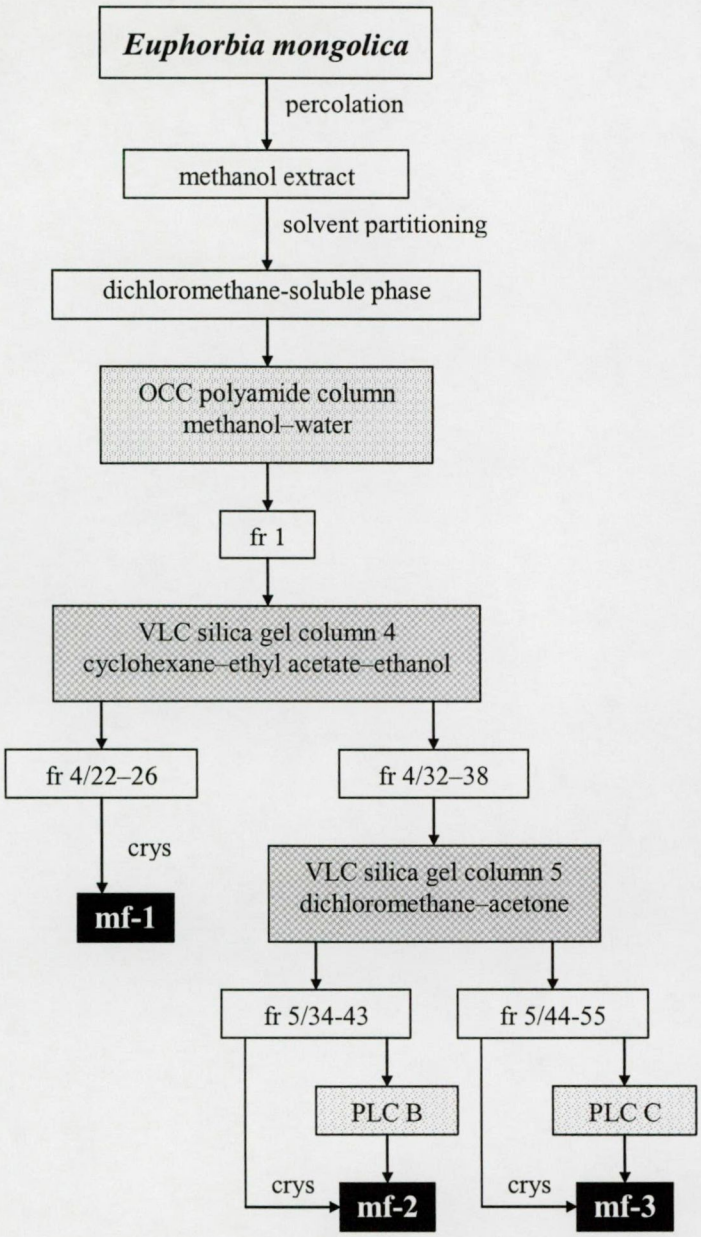


Figure 6. Isolation of diterpenes from *E. mongolica*

4.2. Characterization and structure determination of the isolated compounds

Some physical properties of the isolated compounds are listed in Table 1. The structure elucidation was performed by means of detailed NMR studies, HR-MS measurements, UV and IR spectroscopy and, in a few instances, X-ray crystallography.

IR and UV spectroscopy provided little information on the molecules. The IR absorption bands at 3447 cm^{-1} (eser-1) and at 1745–1720 cm^{-1} (eser-1, eser-3) indicated the presence of hydroxy and C=O groups (ester and keto) in the molecules. The UV spectral data of the compounds were very similar (Table 1). All the spectra exhibited 2 absorption maxima (220–230 nm and 272–274 nm) suggesting the presence of an aromatic group. The HR-MS measurements revealed the exact masses and molecular compositions of the compounds. The fragment ions observed indicated the sequential loss of esterifying acids (acetic, benzoic and pentenoic acids) from the parent ions. The most useful data on the chemical structures of the compounds were obtained from advanced 1D and 2D NMR experiments, including ^{1}H NMR, *J*-modulated spin-echo experiment (JMOD), ^{1}H-^{1}H correlated spectroscopy (COSY), nuclear *Overhauser* effect spectroscopy (NOESY), heteronuclear multiple-quantum coherence (HMQC) and heteronuclear multiple-bond correlation (HMBC) measurements. In the cases of eser-1 and eser-9, X-ray diffraction analysis was performed, and from these experiments the absolute configuration of each stereogenic centre was determined.

Table 1. Yields and physical data of the isolated compounds

	Yield (mg)	UV maxima (nm) (log ε)	Optical rotation in chloroform at 25°C (c)		Melting point (°C)
eser-1	13.0	223.5 (4.18), 273.5 (2.94), 282 sh (2.85)	+135 *	(0.05)	137–139
eser-2	5.9	225 (4.08), 272 (2.71), 281 sh (2.75)	+88	(0.05)	–
eser-3	48.1	230 (4.05), 273.5 (2.94), 280 sh (2.86)	+25 †	(0.055)	193–196
eser-4	10.6	229 (3.91), 274 (2.79), 281 sh (2.69)	–107	(0.2)	–
eser-5	1.0	230 (3.67), 271 (2.91), 281 sh (2.89)	+43	(0.025)	–
eser-6	2.2	230 (3.96), 275 (2.92), 282 sh (2.89)	–15	(0.025)	–
eser-7	5.9	231 (3.75), 275 (2.78), 282 sh (2.72)	–18	(1.5)	–
eser-8	23.9	229 (3.80), 273 (2.73), 281 sh (2.67)	–98	(0.06)	220–223
eser-9	5.2	230 (3.92), 273 (2.80), 281 sh (2.72)	–80	(1.5)	196–199
eser-10	2.3	229 (3.98), 273 (2.83), 281 sh (2.73)	–8	(0.05)	–
eser-11	1.0	229 (3.65), 275 (2.94), 282 sh (2.94)	+34	(0.05)	–
eser-12	1.0	230 (3.90), 273 (2.82), 282 sh (2.75)	–15	(0.05)	–
mf-1	20.4	240 (3.71), 274 (2.93), 282 sh (2.88)	+95	(0.10)	178–180
mf-2	13.9	240 (3.69), 274 (2.93), 281 sh (2.87)	+31	(0.175)	198–202
mf-3	7.3	240 (3.66), 274 (2.91), 281 sh (2.85)	+64	(0.20)	198–199

* t = 28.5 °C
† t = 28 °C

Eser-1

The molecular formula of eser-1 was assigned as $C_{38}H_{48}O_{12}$ via ESIMS and NMR investigations. The ^1H NMR and JMOD spectra indicated the presence of 1 benzoyl, 1 tigloyl and 3 acetyl groups (Table 1 in Appendix I). Further analysis of the ^1H and ^{13}C NMR resonances revealed that eser-1 contains 1 disubstituted (δ_C 139.7 and 135.3, δ_H 5.41 d and 5.27 dd) and 1 trisubstituted (δ_C 133.5 and 140.5, δ_H 5.41 d) carbon-carbon double bond. From the molecular formula, a degree of unsaturation of 11 was deduced, which required the presence of a tricyclic skeleton. The JMOD experiment confirmed a 20 carbon-containing diterpene skeleton involving 4 methyls, 1 methylene and 10 methine groups and 5 quaternary carbons. The ^1H-^1H COSY and HMQC experiments indicated 3 structural fragments of correlated hydrogen atoms: δ_H 2.56 dd, 1.76 dd, 2.45 m, 0.93 d and 6.23 brd [–CH_2–$CH(CH_3)$–CH(OR)– (R = acyl)] (A); δ_H 5.79 d and 5.59 d [–CH(OR)–CH(OR)–] (B); δ_H 5.41 d, 5.27 dd, 3.15 m, 0.96 d and 4.96 d [–CH=CH–$CH(CH_3)$–CH(OR)–] (C). Further, in the ^1H-^1H COSY spectrum, weak ^4J H-H coupling was detected between the hydrogen atoms at δ_H 6.23 and 5.41 d (J = 1.7 Hz), indicating their close proximity.

The sequences A, B and C, tertiary methyls and quaternary carbons were connected by means of an HMBC experiment (Figure 7). The long-range correlations of the quaternary carbons at δ_C 91.5 (C-15) and 133.5 (C-4) with the signals at δ_H 2.56, 1.76 (H-1α, β), 6.23 (H-3) and 5.41

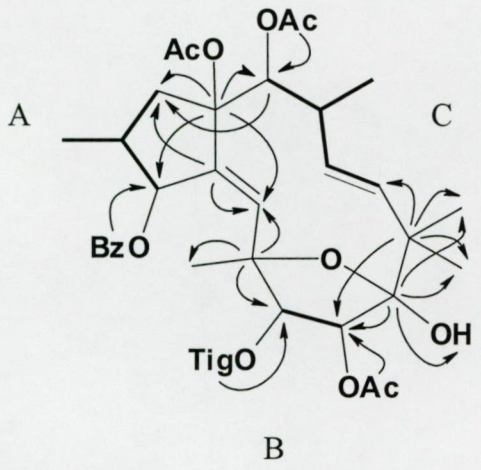


Figure 7. Selected ^1H-^1H COSY (▬) and HMBC (→) correlations for eser-1

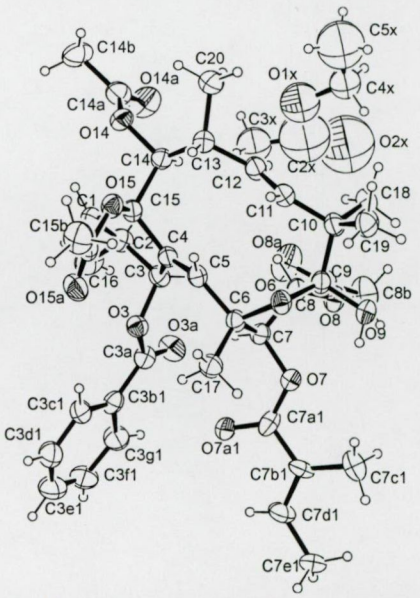
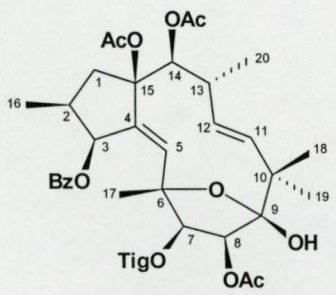
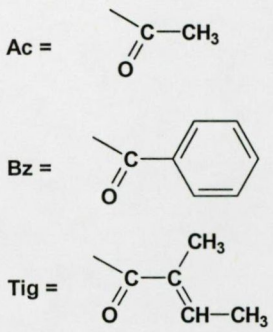


Figure 8. Perspective view of eser-1, using thermal ellipsoids with a 30% probability level

(H-5) revealed that structural element A, together with 2 quaternary carbons, forms a methyl-substituted 5-membered ring, present in many types of Euphorbiaceae diterpenes. HMBC cross-peaks between the signals at δ_C 82.7 (C-6) and δ_H 5.41 (H-5), 5.79 (H-7) and 1.13 (H-17) indicated the linkage of structural part B and 1 methyl group. The couplings $^2J_{C,H}$ and $^3J_{C,H}$ of the carbons at δ_C 105.5 (C-9) and 42.8 (C-10) and the hydrogen atoms at δ_H 5.59 (H-8), 5.41 (H-11), 5.27 (H-12), 1.18 (H-18) and 0.91 (H-19) clearly suggested that the molecular moieties B and C and 2 methyl groups are connected through quaternary carbons. Inspection of the correlations between δ_C 91.5 (C-15) and δ_H 4.96 (H-14) and between δ_C 76.1 (C-14) and δ_H 2.56, 1.76 (H-1α, β) revealed that the full structure involved a jatrophane carbon skeleton. The positions of the ester groups were also determined via the HMBC experiment by evaluation of the $^3J_{C,H}$ couplings between the oxymethine hydrogen atoms and the carbonyl carbons. The position of the hydroxy group was evident from the cross-peak between the signals at δ_C 105.5 (C-9) and δ_H 3.34 (OH). The acetyl group (δ_H 2.21), which did not exhibit any C*H*O–*C*OR correlations, must be situated on one of the quaternary carbons. The –*C*–O–CO–C*H*$_3$ couplings ($^4J_{C,H}$ = 2.1±0.1 Hz) observed in the HMBC spectrum fixed the location of this acetyl group at C-15, and of necessity ethereal functions (deduced from the molecular formula) were sited at positions C-6 and C-9. The stereochemistry of eser-1 was determined by means of X-ray diffraction. Figure 8 and structural formula **1** present the absolute configuration.



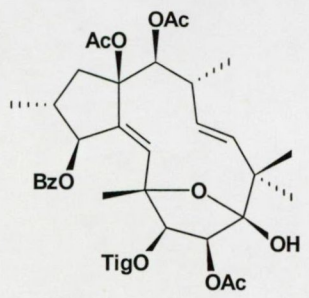
1



Eser-2

The molecular composition $C_{38}H_{48}O_{12}$ of eser-2 was assigned by HRESIMS from the ion with *m/z* 829.2205 $[M + Cs]^+$. The ^1H NMR and JMOD spectral data on eser-2 (Table 1 in Appendix IV) were very similar to those of eser-1 (**1**), differing significantly only in the chemical shifts of H-1α, H-2, H-3, H-16, C-1, C-2, C-3 and C-16. 1D and 2D NMR studies resulted in the same planar structure for eser-2 as that of eser-1. Accordingly, the difference must lie in their stereochemistry.

The relative configuration of eser-2 was investigated by a phase-sensitive NOESY experiment (Table 1 in Appendix IV), aided by consideration of the coupling constant values. Starting from the α position of H-3, it was found that an α-oriented methyl group is present at C-2, and H-14 is in the α position with regard to the NOEs observed between H-3 and H-1α, H-1α and H-14, H-1β and H-2, and H-14 and H-16. The coupling constant $J_{13,14}$ = 9.3 Hz is consistent with a β-oriented H-13, similarly as in eser-1. $J_{11,12}$ = 16.2 Hz required the *E* geometry of the C-11/C-12 olefin linkage. As regards the stereochemistry of the C-8–C-12 part of the molecule, the NOESY correlations between H-13, and H-11, H-11 and H-18, H-12 and H-19, and H-19 and H-8 were informative, indicating that H-12 is directed below, and H-11 above the plane of the macrocycle, and H-8 and H-19 are in the α position. In the absence of appreciable NOEs, the orientation of H-7 was concluded from its coupling constant. The value $J_{7,8}$ = 4.0 Hz indicated an α-oriented H-7, similarly as in the case of eser-1. The NOE interactions between H-3" (tigloyl) and 9-OH, H-3" and H-17, H-17 and H-5, and H-5 and 15-OAc are compatible with β-oriented 9-OH, 17-methyl, 7-tigloyloxy and 15-acetoxy groups. The configuration of the C-4–C-5 double bond was assigned as *E* from the NOESY correlations of H-5 with 15-OAc and H-5 with H-13. As a result of the above NMR study, the structure of eser-2 was elucidated as **2**, which differs from that of eser-1 (**1**) only in the configuration of C-2.



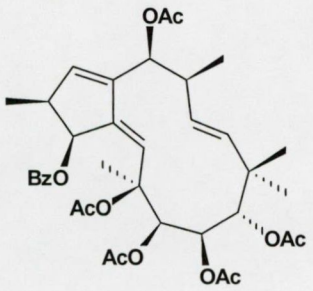
2

Eser-5

Eser-5, an amorphous solid, has the molecular formula $C_{37}H_{46}O_{12}$, determined via the quasimolecular ion peak at *m/z* 815.2043 $[M + Cs]^+$ in the HRESIMS and supported by the hydrogen and carbon atom counts in the NMR spectra. The ^1H NMR spectrum suggested the presence of 1 benzoyl and 5 acetyl groups in the molecule (Table 2 in Appendix IV). The skeletal carbons and directly bonded hydrogen atoms were assigned by means of HMQC, ^1H-^1H COSY, total correlated spectroscopy (TOCSY) and HMBC experiments. Excluding the resonances of ester moieties, the signals of 5 methyls, 5 oxygen-substituted and 2 alkyl-substituted methines, 4 tertiary unsaturated carbons, 1 oxygen-substituted, 1 alkyl-substituted and 2 unsaturated quaternary carbons were observed in the spectrum. Thus, eser-5 is based on a diterpenoid skeleton with 3 carbon-carbon double bonds and 6 ester functions. Interpretation of the ^1H-^1H COSY and TOCSY spectra revealed the existence of 3 partial structures with regard to the cross-peaks between the signals at δ_H 5.87 s, 3.29 dq, 1.02 d and 6.15 d [=CH–CH(CH_3)–CH(OR)–] (A), 5.30 d, 5.49 d and 5.41 s [–CH(OR)–CH(OR)–CH(OR)–] (B) and 5.40 d, 5.64 dd, 2.42 dq, 1.14 d and 5.64 s [–CH=CH–CH(CH_3)–CH(OR)–] (C). After the chemical shifts of the protonated carbons had been assigned via the HMQC spectrum, the connection of these 3 fragments and 4 quaternary carbons was performed on the basis of the HMBC spectrum. The long-range correlations led to the conclusion that eser-5 is a jatrophane triene substituted with ester groups at C-3, C-6, C-7, C-8, C-9 and C-14. From the HMBC correlation between H-3 and the carbonyl carbon of the benzoyl group, it was evident that the benzoate group is present on C-3, and consequently acetyl groups are connected at all other positions.

The relative configuration of eser-5 was assessed by analysis of the coupling constant pattern and the results of the NOESY experiment. As reference point, the position of H-3 was chosen to be α. The observed NOESY correlations between H-3 and H-2, H-3 and H-7, and H-3 and H-17 proved the β position of C-16 and the benzoyl group, and the β orientation of the acetyl groups on C-6 and C-7. The NOE interaction between H-7 and H-11 indicated that H-11 is oriented below the plane of the macrocyclic ring. The cross-peaks of H-11 with H-8, H-13 and H-19 suggested the α position of these hydrogen atoms and the methyl group. H-9 displayed a cross-peak with H-18, and therefore an α-oriented acetyl group must be present at C-9. The coupling constant between H-11 and H-12 (J = 16 Hz) required the *E* geometry of

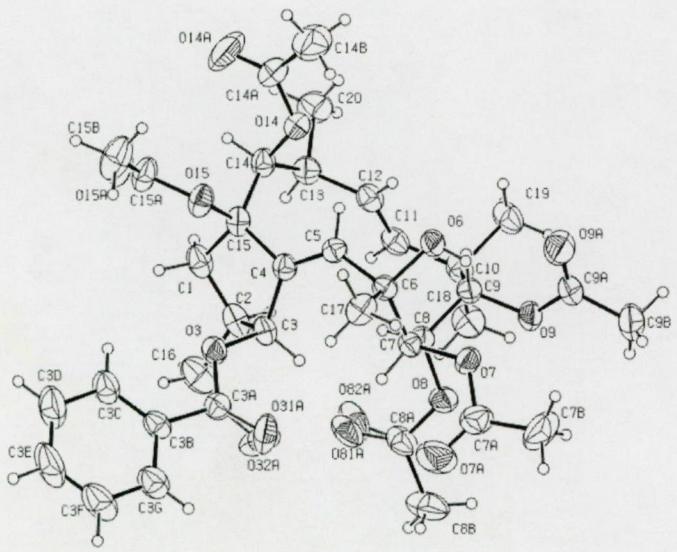
the C-11/C-12 olefin linkage. The C-4/C-5 double bond adopts an *E* configuration, as deduced from the NOESY correlation of H-5 with H-11. All of the above data are compatible with structure **3** for eser-5.

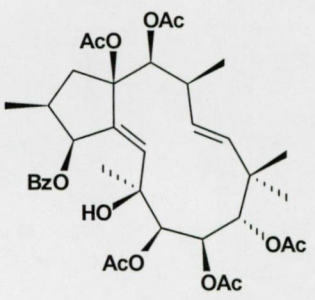


3

Eser-9

The structure and configuration of eser-9 (**4**) were determined by single-crystal X-ray analysis in collaboration with the Institute of Chemistry of the Chemical Research Centre of the Hungarian Academy of Sciences. The perspective view of eser-9 (Figure 9) depicts the absolute stereochemistry of the compound and the atomic numbering applied. A detailed NMR study of eser-9 (**4**), including ^{1}H–^{1}H COSY, NOESY, heteronuclear single quantum correlation (HSQC) and HMBC measurements, resulted in complete, unambiguous ^{1}H and ^{13}C chemical shift assignments (compound **3** in Table 3 in Appendix III).



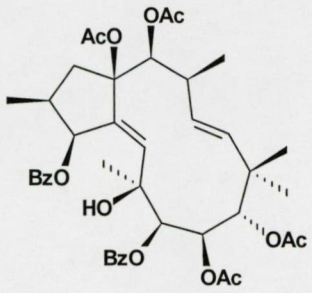


4

Figure 9. Perspective view of eser-9, using thermal ellipsoids with a 30% probability level

Eser-12

Eser-12, isolated in a very small quantity, had the molecular formula $C_{42}H_{50}O_{13}$, as established by HRFABMS. Its NMR spectral data (compound **4** in Table 3 in Appendix III) were very similar to those of eser-9 (**4**) (compound **3** in Table 3 in Appendix III), differing only in the ester residue. In the ^{1}H and ^{13}C NMR spectra of eser-12, signals for 1 acetyl were missing, whereas signals for an additional benzoyl moiety appeared. After the chemical shifts of all carbons and hydrogen atoms had been assigned via the ^{1}H–^{1}H COSY, HSQC and HMBC spectra, it was evident that a benzoate group is present at C-7 in eser-12 because of the observed downfield-shifted H-7 signal [δ_H 6.18 brs (eser-12) and 5.85 brs (eser-9)]. The stereochemistry of eser-12 (**5**) was investigated by NOESY measurement, the results being compared with those on eser-9 (**4**). The NOEs and coupling constants were found to be virtually identical for both compounds, and their conformations in solution were in good agreement with the solid-state conformation determined by X-ray analysis in the case of eser-9 (**4**).



5

Eser-8

Eser-8 was obtained as colourless crystals. Its molecular weight was determined by HRFABMS, indicating the molecular formula as $C_{35}H_{44}O_{12}$. The ^{1}H NMR (Figure 10) and JMOD spectra showed 4 acetyl moieties and 1 benzoyl group (Table 2 in Appendix III). The NMR data and the molecular formula suggested that eser-8 was a bicyclic diterpene containing 1 disubstituted (δ_C 141.6 and 127.2, δ_H 5.47 d and 5.73 dd) and 1 trisubstituted (δ_C 137.9 and 141.6, δ_H 5.99 brs) carbon-carbon double bond. From the ^{1}H-^{1}H COSY and HMQC spectra of eser-8, 3 sequences of correlated hydrogen atoms could be extracted: [–CH_2–$CH(CH_3)$–CHOR– (R = acyl)] (A), [–CHOR–CHOR–] (B) and [–CH=CH–$CH(CH_3)$–] (C).

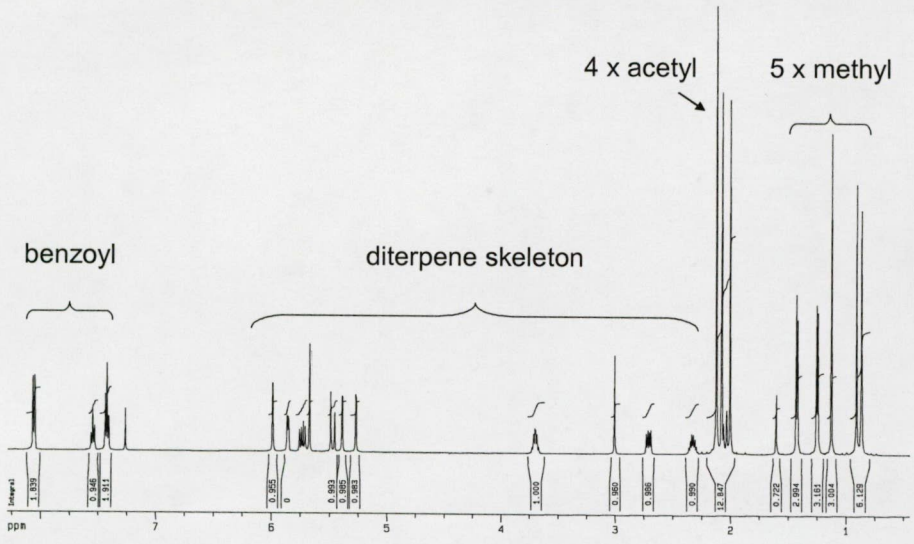


Figure 10. ^{1}H NMR spectrum of eser-8 (500 MHz, $CDCl_3$)

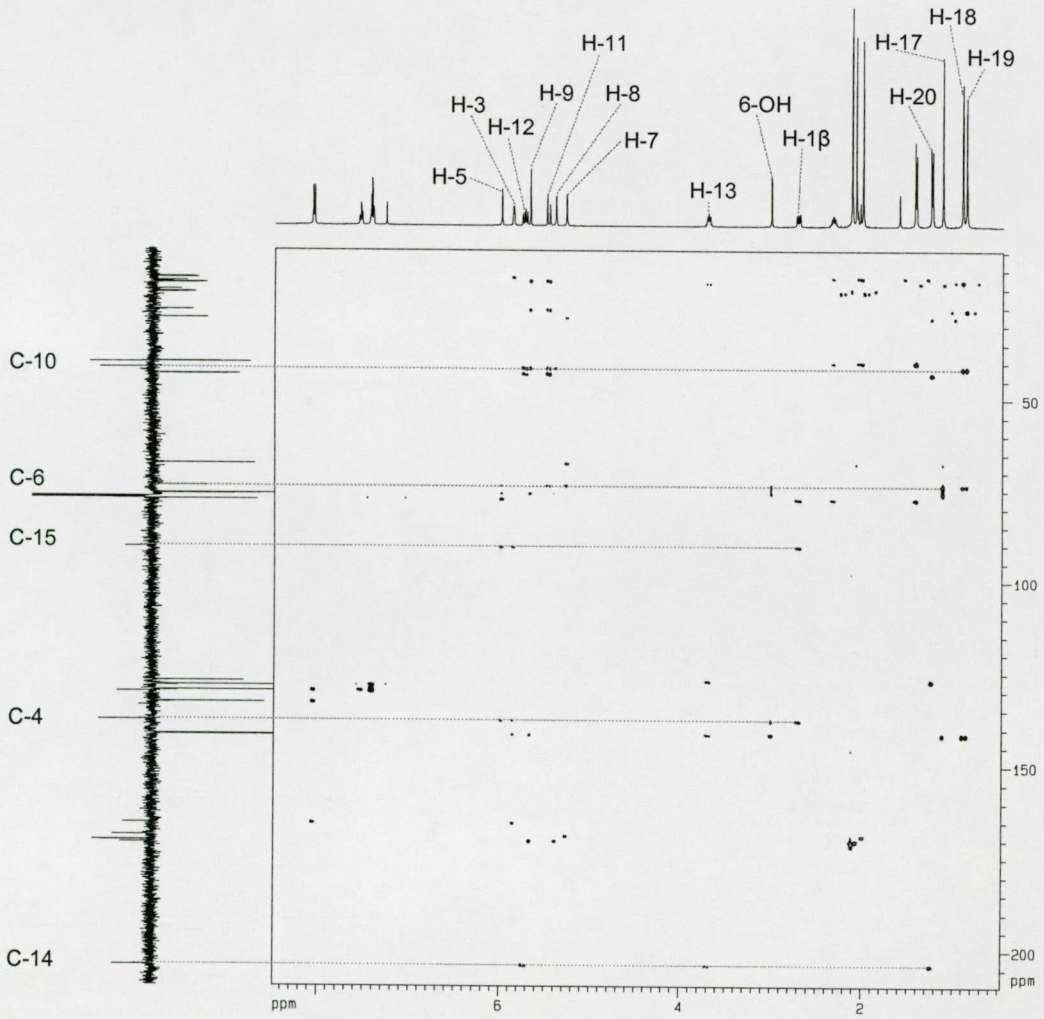
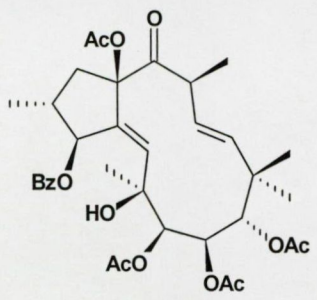


Figure 11. Correlations of quaternary carbons in the HMBC spectrum of eser-8 ($CDCl_3$)

Additionally, in the ^{1}H-^{1}H COSY spectrum, weak $^{4}J_{H,H}$ couplings were detected between the protons at δ_H 5.86 and 5.99 (H-3/H-5), δ_H 5.99 and 3.01 (H-5/6-OH), δ_H 5.99 and 1.13 (H-5/H-17), and δ_H 5.67 and 0.87 (H-9/H-19), indicating their near position. One proton signal (δ_H 3.01 s), which did not exhibit any correlation in the HMQC spectrum, suggested 1 hydroxy group in the molecule. The HMBC correlations (Figure 11) between C-15 and H-1, C-15 and H-3,C-4 and H-1, and C-4 and H-3 suggested that partial structure A and 2 quaternary carbons (C-4 and C-15) were joined to a methyl-substituted 5-membered ring. Similarly, the $^{2}J_{C,H}$ and $^{3}J_{C,H}$ couplings of C-4, C-6, C-9, C-10, C-14 and C-15 demonstrated that units B and C, 3 methyls and 2 methines were connected through quaternary carbons, resulting in the 12-membered macrocycle of a jatrophane skeleton. On the basis of the long-range correlations between the ester carbonyl carbons (δ_C 168.9 and 2 x 170.3) and oxymethine hydrogen atoms (δ_H 5.27 brs, 5.39 brs and 5.67 s), the presence of a benzoyl group on C-3 and acetyl groups on C-7, C-8 and C-9 was evident. The remaining acetyl (δ_C 170.9), which did not exhibit any long-range correlation to any skeletal hydrogen, was of necessity situated on quaternary carbon C-15. The $^{2}J_{C,H}$ coupling between C-6 and the hydroxy group pointed to the hydroxy group being on C-6.

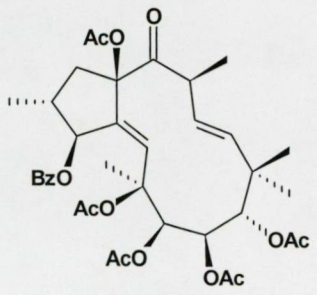
H-3 was taken as reference point for the determination of the stereochemistry and was assigned arbitrarily to the α orientation, as in eser-9 (**4**). The NOEs observed between H-3 and H-1α, H-1α and H-16, H-1β and H-2, H-3 and H-17, H-3 and H-7, H-3 and H-8, and H-1α and H-13 confirmed the α-orientation of these hydrogen atoms. The β configuration of H-9 and the 15-OAc group was deduced from the interactions between 6-OH and H-9 and between the *ortho* benzoyl hydrogen atoms and the 15-OAc group. Further important NOE effects were observed between H-5 and H-11, and H-5 and the 15-OAc group, revealing that H-5 is directed inwards in the macrocyclic ring, and the C-4/C-5 olefin linkage adopts the *E* configuration. The above evidence led to the structure of eser-8 being established as **6**.



6

Eser-3

ESIMS and extensive NMR studies established the molecular formula $C_{37}H_{46}O_{13}$, with the parent ion $[M + Na]^+$ at *m/z* 721. On the basis of its NMR spectral data (Table 2 in Appendix I), eser-3 proved to be a close analogue of eser-8 (**6**) (Table 2 in Appendix III). The difference lay in the number of ester groups. The ^1H NMR spectrum of eser-3 suggested the presence of 5 acetoxy and 1 benzoyloxy groups. The JMOD spectrum displayed 4 methines and 2 quaternary carbons linked to oxygen (δ_C 67.8, 73.1, 76.0, 77.4, 81.8 and 90.6). The downfield-shifted C-6 signal [δ_C 81.8 (eser-3) and 73.7 (eser-8) (**6**)] suggested that eser-3 bears an acetoxy group instead of a hydroxy at C-6. The relative configuration of eser-3 was established on the basis of the NOESY correlations between H-3 and H-1α, H-1α and H-16, H-1α and H-13, H-3 and H-7, H-7 and H-8, H-8 and H-17, H-7 and H-19, H-1β and H-2, H-2 and 15-OAc, H-18 and H-9, H-9 and 6-OAc, H-5 and 15-OAc, and H-5 and H-11. Thus, the structure of eser-3 was determined as **7**.

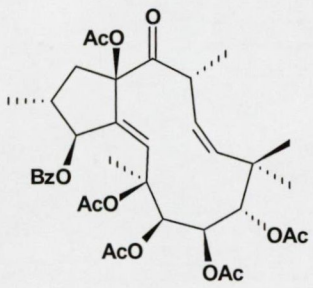


7

Eser-11

Eser-11 was obtained in a very small quantity as an amorphous solid. HRESIMS suggested the molecular formula $C_{37}H_{46}O_{13}$ with the $(M + Na)^+$ ion at *m/z* 721.2838. On evaluation of the 1D and 2D NMR spectra, eser-11 proved to be 6,7,8,9,15-pentaacetoxy-3-benzoyloxyjatropha-4,11-dien-14-one (compound **5** in Table 3 in Appendix IV). Thus, eser-11 and eser-3 (**7**) have the same planar structure, but they differ in some respects in their stereochemistry. The coupling constant $J_{11,12} = 12.8$ Hz revealed the *Z* configuration of the C-11/C-12 double bond. The chemical shifts of H-2, H-3, H-16, C-2, C-3 and C-16 were in good agreement with those of eser-8 (**6**) and eser-3 (**7**), suggesting the β orientation of H-2 and the benzoyl group at C-3. The NOEs between H-3 and H-7, H-3 and H-17, H-7 and H-17, H-7 and H-8, H-8 and H-19, and H-18 and H-9 established the β position of the acetyl groups

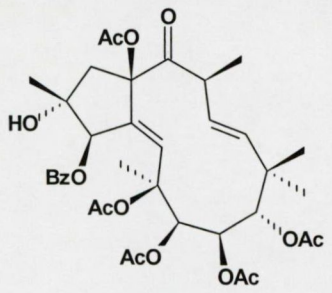
at C-6, C-7 and C-8, and the α orientation at C-9, similarly as in the case of eser-3. However, the NOESY correlations of H-9 with H-13 indicated the α position of the methyl group at C-13. The *E* configuration of the C-5/C-6 olefin linkage was derived from the NOESY correlation between H-5 and H-12. This evidence led to the formulation of eser-11 as **8**.



8

Eser-7

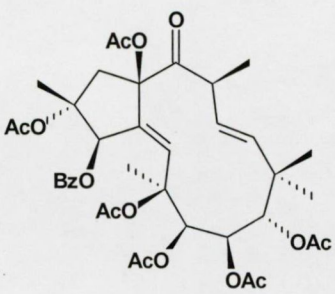
Eser-7, an amorphous solid, has the molecular formula $C_{37}H_{46}O_{14}$, determined via the quasimolecular ion peak at *m/z* 737.2805 $[M + Na]^+$ in the positive HRESIMS. Comparison of the ^1H and ^{13}C NMR chemical shifts of eser-7 with those of eser-8 (**6**) (Table 2 in Appendix III) suggested that eser-7 contained an additional acetoxy group. After the ^1H and ^{13}C NMR signals had been assigned by interpretation of the ^1H–^1H COSY, HSQC and HMBC spectra (compound 2 in Table 3 in Appendix III), it was obvious that the new oxygen function is at C-2, in consequence of the absence of the H-2 signal and the appearance of the C-2 signal. The positions of the ester residues on C-3, C-7, C-8 and C-9 were confirmed through the observation of long-range –C*H*–O*C*OR correlations in the HMBC spectrum. The acetyl and hydroxy groups situated on quaternary carbons (C-2, C-6 and C-15) were located by comparison of the ^{13}C chemical shifts of eser-7 with those of other compounds in the eser series. Acetoxy substitution at C-6 and C-15 [$\delta_{C\text{-}6}$ 81.8 (eser-8) and 81.5 (eser-7); $\delta_{C\text{-}15}$ 90.6 (eser-8 and eser-3) and 91.1 (eser-7)] and hydroxy substitution at C-2 was proved by the downfield-shifted C-6 and C-15, and upfield-shifted C-2 signals [$\delta_{C\text{-}2}$ 80.8 (eser-7) and 89.2 (eser-6 and eser-10)]. On the basis of a NOESY experiment, the stereochemistry of eser-7 was found to be identical with that of eser-8 (**6**), with the exception of C-2. The NOEs between H-13 and H-1α and between H-1β and H-16 demonstrated the β orientation of the methyl group at C-2. These data are compatible with structure **9** for eser-7.



9

Eser-6

Eser-6 has the molecular formula $C_{39}H_{48}O_{15}$, as established by HRESIMS. Its NMR spectral data (compound **4** in Table 3 in Appendix IV) were similar to those of eser-7 (**9**) (compound **2** in Table 3 in Appendix III), differing only in the esterification pattern. In the ^{1}H and ^{13}C NMR spectra of eser-6, the signal of a hydroxy group was missing, and the signal of an additional acetyl group appeared. After the chemical shift assignment of all the carbons and hydrogen atoms via 2D NMR spectra had been achieved, it was evident that in eser-6 an acetyl group is present at C-2, because of the downfield-shifted C-2 signal [$\delta_{C\text{-}2}$ 89.2 (eser-6) and 80.8 (eser-7)]. The relative configuration of eser-6, determined by means of a NOESY experiment and aided by the coupling constant values, proved to be the same as that of eser-7 (**9**). The structure of eser-6 is shown by formula **10**.

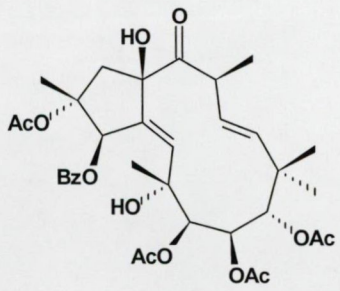


10

Eser-10

Eser-10 was obtained as an amorphous solid with the molecular formula $C_{35}H_{44}O_{13}$. On the basis of ^{1}H and 2D NMR experiments, the constitution of eser-10 (compound **3** in Table 3 in Appendix IV) proved to be very similar to that of eser-6 (**10**) (compound **4** in Table 3 in

Appendix IV). Comparison of the ^1H and JMOD spectra of eser-6 and eser-10 suggested that eser-10 bears 2 free hydroxy functions. These groups can be located at C-6 and C-15 with respect to the $^2J_{C,H}$ couplings observed in the HMBC spectrum between C-6 and the hydrogen signal at δ_H 4.01, and between C-15 and the hydrogen at δ_H 4.58. The relative configuration of eser-10, determined via a NOESY experiment, was found to be very close to that of eser-6 and eser-7. There was a difference in the configuration of C-6, since the NOE correlations between H-3 and 6-OH, and between 6-OH and H-8, were indicative of the α orientation of the hydroxy group at C-6. These data are compatible with structure **11** for eser-10.

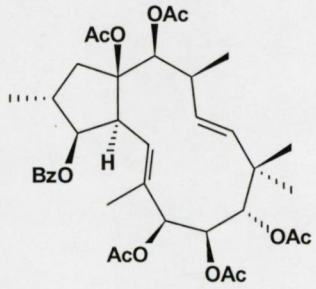


11

Eser-4

Eser-4, obtained as an amorphous solid with the molecular formula $C_{35}H_{46}O_{11}$, was shown on the basis of a detailed NMR study to be a polyacylated derivative of a jatrophane diene with 1 benzoate and 4 acetate groups positioned at C-3, C-8, C-9, C-14 and C-15,. The ^1H–^1H COSY spectrum of eser-4 revealed the presence of a structural fragment –CH_2–CH(CH_3)–CHOR–CH–CH=, which was assigned with the aid of the HMBC spectrum as the C-1–C-5 part of the molecule. Thus, in this compound the olefin linkage is between C-5 and C-6, in contrast with the other compounds obtained from *E. serrulata*. Such compounds, including the anticancer-active euphornin, were isolated earlier from *E. helioscopia* and *E. maddeni*.[85,89,126] The configuration of eser-4 was deduced from the NOESY experiment and from the coupling constant patterns. Starting from the H-4α reference point[89], NOEs between hydrogen pairs H-4/H-13, H-4/H-14, H-14/H-1b, H-1b/H-3 and H-8/H-19 were indicative of the α orientation of these hydrogen atoms. NOEs between hydrogen pairs H-1a/H-2, 15-OAc/H-9, 15-OAc/H-18, H-9/H-18 and H-9/7-OH required their β position. The *E* geometry of the C-11/C-12 olefin linkage followed from the coupling constant of $J_{11,12}$ = 16 Hz, while the *E*

configuration of the C-5/C-6 olefin linkage was concluded from the NOEs detected between H-4/H-17 and H-5/15-OAc. As concerns the conformation of the C-11/C-12 olefin linkage, NOESY cross peaks between H-11/H-13, H-11/H-19, H-12/H-18 and H-12/H-9 were informative, indicating that H-11 is directed below, and H-12 above the plane of the macrocycle. For eser-4, structure **12** was elucidated.

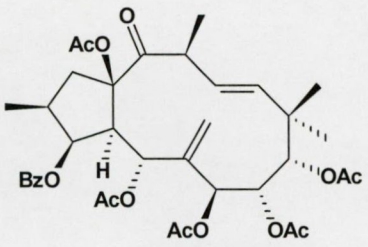


12

Mf-3

Mf-3 was isolated as colourless crystals with the molecular formula $C_{37}H_{46}O_{13}$, as indicated by the quasimolecular ion peak at *m/z* 831.1983 $[M + Cs]^+$ in the HRESIMS spectrum. From the ^1H NMR and JMOD spectra, 6 ester residues were identified as 1 benzoate and 5 acetate groups (compound **2** in Table 1 in Appendix V). The diterpene core was found to contain 1 keto function, 1 olefin group (δ_C 135.9 and 130.5 ppm) and 1 *exo*-methylene. [106,107,111] On the basis of the HMBC correlations between the carbonyl carbons and the ester-bearing methine hydrogen atoms, the benzoyl group was placed at C-5, and the acetyl groups at C-3, C-7, C-8 and C-9. The fifth acetyl group (δ_H 2.18 s), situated on quaternary carbon C-15, was allocated via the *C*-O-CO-C*H*$_3$ 4-bond coupling, observed as a weak signal in the HMBC spectrum. The position of the keto group at C-14 was evident from the heteronuclear long-range correlations of the signal at δ_C 204.2 with H-1, H-4, H-12, H-13 and H-20. The stereochemical aspects were studied by means of a NOESY experiment. NOEs were detected between hydrogen pairs H-4/H-2, H-4/H-3, H-4/H-7, H-3/H-7, H-4/H-13 and H-13/H-1α, suggesting the α orientation of these hydrogen atoms. It was found that H-11 is directed below, and H-12 above the plane of the macrocycle, while H-19 is in the α and H-18 in the β position with regard to the NOESY correlations between H-11/H-13, H-11/H-19 and H-12/H-18. Moreover, the NOEs between H-18 and H-8, and between H-12 and H-5,

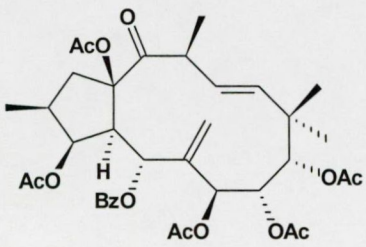
demonstrated β-oriented H-8 and H-5. Further important NOEs of the ester groups were observed, such as H-7/9-OAc and H-2',6'/15-OAc, which provided evidence of the 15β and 9α configurations of the acetyl groups. In conclusion, the structure of mf-3 was elucidated as **13**.



13

Mf-2

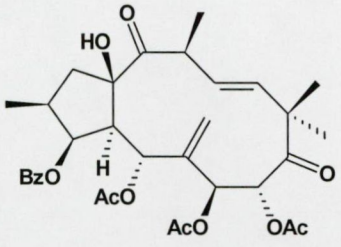
Mf-2 was crystallized from methanol as white crystals. It possessed the same molecular formula as mf-3. The ^{1}H NMR and JMOD spectra of mf-2 (compound **3** in Table 1 in Appendix V) were very similar to those of mf-3 (**13**), indicating the presence of the same diterpene polyol and ester groups, and differing only in the esterification pattern. Comparison of the ^{1}H NMR data on mf-3 and mf-2 revealed that the C-5 acetyloxy moiety was replaced by a benzoyloxy group with respect to the downfield-shifted H-5 signal [$\delta_{H\text{-}5}$ 5.41 (mf-3) and 5.65 (mf-2)]. Additionally, the presence of the acetyl groups at C-3, C-7, C-8 and C-9 was suggested by the heteronuclear long-range correlations of the carbonyl ^{13}C signals. The NOEs demonstrated the same configuration of mf-2 as that of mf-3 (**13**). These observations permitted the assignment of this compound as **14**.



14

Mf-1

Mf-1 was obtained as colourless crystals. The HRESIMS data indicated the molecular formula $C_{33}H_{40}O_{11}$ on the basis of the $[M + Cs]^+$ ion at *m/z* 745.1630. The ^1H NMR and JMOD spectra showed similarities to those of mf-3 (**13**), but mf-1 contained 3 acetate, 1 benzoate and 2 keto groups (δ_C 212.3 and 204.2 ppm) (compound **1** in Table 1 in Appendix V). One hydrogen signal (δ_H 4.26 s), which did not exhibit any correlation in the HMQC spectrum, suggested the presence of 1 hydroxy group in the molecule. The long-range correlations of the carbonyl carbons (δ_C 170.3, 169.9, 168.8 and 165.8) and oxymethine protons (δ_H 5.93, 5.35, 5.36 and 5.83) clearly showed the presence of the acetyl groups at C-5, C-7 and C-8, and the benzoyl group at C-3. The position of the keto group at C-9 was evident from the heteronuclear long-range correlations of the signal at δ_C 204.5 with H-12, H-11, H-8, H-7, H-18 and H-19. A hydroxy group could be located at C-15 with respect to the HMBC correlation between C-15 and the hydrogen signal at δ_H 4.26. Diagnostic NOE correlations were detected between hydrogen pairs H-4/H-2, H-4/H-3, H-4/H-7, H-4/H-13, H-3/5-OAc, H-4/H-11, H-11/H-19, H-12/H-18, H-18/H-8, H-5/H-17b, H-17b/15-OH, 15-OH/H-1β and H-1β/H-16. This led to the conclusion that 5-OAc, 8-OAc, 19-Me and H-11 are on the α face, while 2-Me, 3-OBz, 7-OAc, 18-Me, H-12, 13-Me and 15-OH are on the β face of the molecule (**15**).



15

5. Discussion

The spurges are a source of structurally diverse isoprenoids such as diterpenoids, triterpenoids and polyterpenoids. Besides the well-known skin-irritant and tumour-promoting phorboids, numerous bioactive diterpenes with different macrocyclic skeletons have been isolated from various Euphorbiaceae species. The structural variability of these compounds is further amplified by esterification with a diverse array of acids. As in many others plant families, in the Euphorbiaceae the taxonomic border at a subfamilial level is an unresolved problem, and a contribution to a better understanding of this could be made by studies of the structural diversity of their diterpenes. Although many compounds with marked biological activity have been isolated from many *Euphorbia* species and studied since the 1960s, only scanty data is available on the chemical constituents of the *E. serrulata* occurring in the northern hemisphere or on the Asian species *E. mongolica*. We have investigated apolar extracts of both plants in order to acquire new knowledge on the diterpenes of these species and to obtain pure compounds for biological and pharmacological experiments.

Diterpenes have previously been detected in various organs of Euphorbiaceae species: in the roots[109], seeds[37], latex[127,128] and aerial parts[111,129]. Thus, the whole plant of *E. serrulata* and the aerial parts of *E. mongolica* (all the available plant material) were extracted so as to obtain the best possible yield of diterpenes. Since the Euphorbiaceae species usually contain these compounds only in low concentrations, the diterpenes are not detectable directly in the crude extracts. The screening method for diterpene content therefore includes a three-step sample preparation procedure. In the cases of *E. serrulata* and *E. mongolica*, evaluation of the screening led to the conclusion that a number of diterpene esters are present, which can be enriched in the 60% aqueous methanol fraction of the apolar extract.

The isolation of diterpene polyesters is a demanding procedure, because these compounds occur merely in low quantities in plants, and often as complex mixtures of esters with structurally related nuclei or the same nucleus. The components may display very similar chromatographic characters, and their separation therefore requires the use of a multi-step method. The ester character of the molecules precludes the application of strongly acidic or basic agents.

The plant material was extracted with methanol, which is an inexpensive amphipolar solvent suitable for the extraction of lipophilic and polar compounds from frozen fresh plant material

with a high water content (*E. serrulata*). In the initial step of separation, liquid-liquid extraction was applied to remove the polar constituents. Thereafter, a specific purification method was used (OCC on polyamide), resulting in a diterpene fraction free from chlorophyll, triterpenes and fats. Repeated VLC separations of the diterpene fraction afforded subfractions containing only a few main components. The degree of purity and the degree of complexity of the fractions determined the final purification method. The relatively complex sub-fractions were subjected to NP and RP HPLC purification. This expensive on-line preparation technique resulted in the isolation of numerous constituents under mild conditions. The PLC method was applied for the purification of large fractions containing 1–2 main components. Successful separations were achieved when different stationary and mobile phases were used in the subsequent steps of purification.

After extensive chromatographic purifications 12 compounds (eser-1–12) were isolated from *E. serrulata*, and 3 (mf-1–3) from *E. mongolica*. The total yield of eser-1–12 was 120.1 mg (0.005% of the fresh plant material), and that of mf-1–3 was 43.7 mg (0.026% of the dry plant material). The solubility, lipophilicity and chromatographic behaviour of the compounds suggested their diterpene ester character.

The structures of the isolated compounds were elucidated by means of spectroscopic methods. The IR, UV and HR-MS spectra provided information about the presence of hydroxy, keto and ester groups. The most useful data concerning the chemical structures of the compounds came from advanced 1D and 2D NMR spectroscopy, including ^{1}H NMR, JMOD, ^{1}H-^{1}H COSY, NOESY, HMQC and HMBC experiments. As a result of detailed NMR studies, complete ^{1}H and ^{13}C chemical-shift assignments of all compounds were made. Their relative configurations and conformations in solution were analysed on the basis of NOESY measurements. The absolute configurations of eser-1 (**1**) and eser-9 (**4**) were determined by X-ray crystallography.

The isolated compounds were identified as highly functionalized jatrophane diterpenes based on 9 novel and 2 known parent alcohols. All the isolated compounds (**1–15**) are new natural products and display considerable structural diversity.

The diterpenes of *E. serrulata* differ in many respects: the number and positions of double bonds and oxygen functions, the nature and location of the ester moieties, and the configurations of the stereogenic centres. Eser-1–3 and eser-5–12 (**1–11**) are the first known compounds of the group of jatrophanes unsaturated at position C-4/C-5. Eser-1 (**1**) and eser-2

(**2**) have a new heterocyclic ring system because of the C-6–C-9 ethereal function. These two compounds are additional members of the small group of jatrophane polyesters esterified with tiglic acid. Eser-5 (**3**) is the first jatrophane diterpene with a double bond in the 5-membered ring. The olefin linkage in eser-4 (**12**) is at C-5/C-6, in contrast with the other compounds in the eser series. Eser-3 (**7**) (48.1 mg) and eser-8 (**6**) (23.9 mg) exhibited close structural similarity and proved to be the main jatrophane constituents of *E. serrulata*. Eser-6–7 and eser-10 (**9–11**) contain 8 oxygen functions, and thus are the highest oxygenated compounds isolated. The diterpenes of *E. serrulata* demonstrate great stereochemical diversity. In eser-2–4, eser-8 and eser-11 (**2, 6–8, 12**), an α-oriented methyl group is present at C-2, but in eser-1, eser-5–7, eser-9–10 and eser-12 (**1, 3–5, 9–11**) this methyl group is in the β position. Eser-1–2 and eser-11 (**1, 2, 8**) contain an α-oriented methyl group at C-13, while in the other compounds this methyl group is in the β position. In eser-10 (**11**), the orientation of the 17-methyl group is β, in contrast with eser-3, eser-5–9 and eser-11–12 (**3–10**), which contain an α methyl group at C-6. Eser-11 (**8**) is the only jatrophane diterpene which possesses a C-11/C-12 double bond with *Z* configuration.

The compounds isolated from *E. mongolica* are members of the usual group of jatrophanes containing a C-11/C-12*E* double bond and an exocyclic 6(17)-methylene group. Mf-2 and mf-3 (**13, 14**) are structural isomers and have the same parent alcohol as the diterpene esters isolated from *E. turczaninowii*[106], *E. terracina*[107], *E. altotibetic*[91] and *E. dendroides*[130]. Mf-1 (**15**) is also an ester of a known diterpene alcohol.[106,107] Mf-1–3 (**13–15**) form a stereochemically homogeneous series.

Morphologically, *E. serrulata* and *E. platyphyllos* are closely related and can be distinguished only by comparison of the tubercles of the capsules.[119,120] Our results reveal that the near relationship is manifested in their chemism. The compounds obtained from *E. serrulata* are structurally very similar to those isolated from *E. platyphyllos* in 2003.[131] Both species contain jatrophanes uniquely unsaturated at C-4/C-5, and one of the diterpene esters of *E. platyphyllos* possesses the same new heterocyclic ring system as that of eser-1–2 (**1, 2**). Eser-8 (**6**) is a common constituent of these two *Euphorbia* species. These observations are of chemotaxonomic importance and support the close botanical relationship of the two spurge species.

The isolated compounds were tested for their multidrug resistance (MDR) reversing activity. Resistance to multiple anticancer drugs is one of the main reasons for treatment failure in the

chemotherapy of malignant tumours. A primary mechanism of MDR is the overproduction of permeability glycoprotein (P-gp) in plasma membranes of resistant cells, where the P-gp acts as an energy-dependent efflux pump, reducing the intracellular accumulation of anticancer drugs. Resistance mediated by P-gp is of special importance because the exposure of cancer cells to one cytostatic agent results in a specific resistance not only to the inducer, but also to many other chemically unrelated anticancer drugs, representing a non-specific cross-resistance.[132,133]

In recent years, considerable attention has been devoted to the development of new effective anti-MDR agents from natural sources. In the course of the research programme of the Department of Medicinal Microbiology at the University of Szeged, the team of Professor József Molnár has investigated eser-1–4, eser-7–9 and mf-1–3, using the rhodamine 123 exclusion test described in Appendices III and V; the results are summarized in Table 2. All of the tested compounds displayed a significant effect in inhibiting the efflux-pump activity of multidrug-resistant L-5178 mouse lymphoma cells as compared with that of the positive control verapamil. Within the set of tested compounds, eser-1 and eser-2 exhibited activity many times higher than that of verapamil, and appear to be the most powerful P-gp inhibitors. These results suggested that jatrophane diterpenes form a new family of potent modulators of MDR. Compounds of eser and mf series and several others diterpenes isolated from *Euphorbia* species may be objects of structure-activity relationship studies of the MDR-reversing effect.

Table 2. Reversal of the MDR of mouse lymphoma cells by some isolated diterpenes [the fluorescence activity ratio of verapamil (c = 5 μg/ml) was 8.27 (eser-1–4 and eser-7–9) or 13.14 (mf-1-3)]

		eser-1	eser-2	eser-3	eser-4	eser-7	eser-8	eser-9	mf-1	mf-2	mf-3
Fluorescence activity ratio	**c = 4 μg/ml**	45.14	55.14	10.57	20.98	8.75	8.87	4.31	12.29	2.60	2.79
	c = 40 μg/ml	138.30	58.08	16.08	34.79	12.73	6.70	4.86	22.92	18.02	29.29

The cytotoxicities and antiviral effects of 9 diterpene polyesters, including eser-3, were tested in the Department of Medicinal Microbiology at the University of Szeged (Appendix II). Among the investigated compounds, eser-3 was the least potent cytotoxic agent in Vero cells (CC_{50} 105.8 μg/ml). It moderately reduced the yield of *Herpes simplex* virus type 2 (IC_{50} 7.2 μg/ml) and exhibited the highest selectivity index ($SI = CC_{50}/IC_{50} = 14.7$).

6. Summary

The scope of the present work covered the isolation and structure determination of diterpenes from *Euphorbia serrulata* and *E. mongolica*, whose terpenoid constituents have not been investigated previously.

The preliminary screening of these *Euphorbia* species showed that extracts of *E. serrulata* and *E. mongolica* contain numerous diterpene esters, and that these compounds can be enriched by polyamide OCC in the 60% aqueous methanol fraction of the apolar extract.

From the methanol extracts of *E. serrulata*, 12 compounds (eser-1–12) were isolated and from *E. mongolica*, 3 compounds (mf-1–3) were obtained, through the use of an extensive multi-step separation and purification procedure including OCC, VLC, NP HPLC, RP HPLC and PLC. The structures of the compounds were elucidated by means of detailed NMR experiments, HR-MS measurements, UV spectroscopy and, in a few instances, X-ray crystallography studies and IR spectroscopy.

The isolated compounds (**1–15**) were identified as highly functionalized macrocyclic diterpene based on 9 novel and 2 known parent alcohols. All these compounds are new natural products and reveal the great structural diversity of jatrophanes. Eser-1–3 and eser-5–12 (**1–11**) are the first known jatrophanes unsaturated at position C-4/C-5. Eser-1 (**1**) and eser-2 (**2**) have a new heterocyclic ring system, because of the C-6–C-9 ethereal function. Eser-5 (**3**) is the first known jatrophane diterpene with a double bond in the 5-membered ring. The compounds isolated from *E. serrulata* (**1–12**) exhibit considerable stereochemical diversity. The jatrophanes isolated from *E. mongolica* (**13–15**) are esters of known diterpene alcohols.

Structurally, the compounds obtained from *E. serrulata* are very similar to those isolated from *E. platyphyllos*. These observations are of chemotaxonomic importance and support the close botanical relationship of the two spurge species.

The biological activities of some of the isolated compounds were investigated in the Department of Medicinal Microbiology, University of Szeged. Eser-1–4, eser-7–9 and mf-1–3 exerted pronounced inhibitory effects on the efflux-pump activity of multidrug-resistant L-5178 mouse lymphoma cells as compared with that of the positive control verapamil. Eser-3 displayed low cytotoxicity in Vero cells and a moderate antiviral effect against *Herpes simplex* virus type 2, with a high selectivity index.

7. References

[1] Webster GL, *Bot. J. Linn. Soc.* 1987; **94**: 3-46

[2] Scholz A, Euphorbiaceae. In: Engler A ed. *Syllabus der Planzenfamilien*, Vol. 2, Berlin-Nikolassee: Gebrüder Borntraeger 1964. pp 255-261.

[3] Evans WC, *Pharmacognosy*, Edinburgh, London, New York, Philadelphia, St Luis, Sidney, Toronto: W. B. Saunders 2002. p 27.

[4] Schultes RE, *Bot. J. Linn.* 1987; **94**: 79-95

[5] Hartwell JL, *Lloydia* 1969; **32**: 153-205

[6] Hecker E, Schmidt R, Phorbolesters – the irritants and cocarcinogens of *Croton tiglium* L. In: Herz W, Grisebach H, Kirby GW, eds. *Progress in the Chemistry of Organic Natural Products*, Vol 31, Wien, New York: Springer 1974. pp 377-403.

[7] Evans FJ, Taylor SE, Pro-inflammatory, tumour-promoting and anti tumour diterpenes of the plant families Euphorbiaceae and Thymeleaceae. In: Herz W, Grisebach H, Kirby GW, eds. *Progress in the Chemistry of Organic Natural Products*, Vol 44, Wien, New York: Springer 1983. pp 1-99.

[8] Kupchan SM, Siegel CW, Matz MJ, Renauld JAS, Haltiwanger RC, Bryan RF, *J. Am. Chem. Soc.* 1970; **92**: 4476-4477

[9] Helmboldt H, Rehbein J, Hiersemann M, *Tetrahedron Lett.* 2004; **45**: 289-292

[10] Forgo P, Kövér KE, Hohmann J, *Monatsh. für Ch.* 2002; **133**: 1249-1261

[11] Shizuri Y, Ohtsuka J, Kosemura S, Terada Y, Yamamura S, *Tetrahedron Lett.* 1984; **25**: 5547-5550

[12] Manners GD, Davis DG, *Phytochemistry* 1987; **26**: 727-730

[13] Miranda FJ, Alabadí JA, Ortí M, Centeno JM, Piñón M, Yuste A, Sanz-Cervera JF, Marco A, Alborch E, *J. Pharm. Pharmacol.* 1998; **50**: 237-241

[14] Hohmann J, Vasas A, Günther G, Máthé I, Evanics F, Dombi G, Jerkovich G, *J. Nat Prod.* 1997; **60**: 331-335

[15] Günther G, Hohmann J, Vasas A, Máthé I, Dombi G, Jerkovich G, *Phytochemistry* 1998; **47**: 1309-1313

[16] Hohmann J, Evanics F, Vasas A, Dombi G, Jerkovich G, Máthé I, *J. Nat. Prod.* 1999; **62**: 176-178

[17] Hohmann J, Vasas A, Günther G, Dombi G, Blazsó G, Falkay G, Máthé I, Jerkovich G, *Phytochemistry* 1999; **51**: 673-677

[18] Hohmann J, Evanics F, Berta L, Bartók T, *Planta Med.* 2000; **66**: 291-294

[19] Hänsel R, Sticher O, Steinegger E, *Pharmakognosie – Phytopharmazie*, 6th edition, Berlin, Heidelberg, New York: Springer 1999. pp 495-505.

[20] Schmidt RJ, *Bot. J. Linn. Soc.* 1987; **94**: 221-230

[21] Dewick PM, *Nat. Prod. Rep.* 1997; **14**: 111-144

[22] Bruneton J, *Pharmacognosy, Phytochemistry, Medicinal Plants*, Londres, Paris, New York: Intercept, Technique & Documentation, Lavoisier 1999. pp 637-659.

[23] Roengsumran S, Achayindee S, Petsom A, Pudhom K, Singtothong P, Surachetapan C, Vilaivan T, *J. Nat. Prod.* 1998; **61**: 652-654

[24] Tanaka CMA, Sarragiotto MH, Zukerman-Schpector J, Marsaioli AJ, *Phytochemistry* 1997; **44**: 1547-1549

[25] Duh C-Y, Wang S-K, Tseng H-K, Sheu J-H, Chiang MY, *J. Nat. Prod.* 1998; **61**: 844-847

[26] Rodríguez AD, Acosta AL, *J. Nat. Prod.* 1997; **60**: 1134-1138

[27] Rashid MA, Gustafson KR, Boyd MR, *J. Nat. Prod.* 2000; **63**: 531-533

[28] Kong L-Y, Li Y, Wu X-L, Min Z-D, *Planta Med.* 2002; **68**: 248-252

[29] Xu Z-H, Sun J, Xu R-S, Qin G-W, *Phytochemistry* 1998; **49**: 149-151

[30] Moura VLA, Monte FJO, *J. Nat. Prod.* 1990; **53**: 1566-1571

[31] Ghisalberti EL, Jefferies PR, Mori TA, Skelton BW, White AH, *Tetrahedron* 1985; **12**: 2517-2526

[32] Choi Y-H, Kim J, Pezzuto JM, Kinghorn AD, Farnsworth NR, *Tetrahedron Lett.* 1986, **27**: 5795-5798

[33] Choi Y-H, Pezzuto JM, Kinghorn AD, Farnsworth NR, *J. Nat. Prod.* 1988; **51**: 110-116

[34] Hecker E, *Cancer Res.* 1968; **28**: 2338-2349

[35] Adolf W, Hecker E, *Tetrahedron Lett.* 1970; **26**: 2241-2244

[36] Hecker E, Adolf W, Kubinyi H, *Tetrahedron Lett.* 1970; **35**: 3071-3073

[37] Onwukaeme NO, Rowan MG, *Phytochemistry* 1992; **31**: 3479-3482

[38] Upadhyay RR, Hecker E, *Phytochemistry* 1975; **14**: 2514-2515

[39] Sosath S, Ott HH, Hecker E, *J. Nat. Prod.* 1988; **51**: 1062-1074

[40] Abo K, Evans FJ, *J. Pharm. Pharmacol.* 1981; **33**: 57

[41] Abo K, Evans FJ, *Phytochemistry* 1981; **20**: 2535-2537

[42] Mirinda FJ, Alabadí JA, Pérez P, Ortí M, Centeno JM, Yuste A, San-Cervera JF, Marco A, Alborch E, *J. Pharm. Pharmacol.* 1997; **49**: 573-576

[43] Miranda FJ, Alabadí JA, Ortí M, Centeno JM, Piñón M, Yuste A, Sanz-Cervera F, Marco A, Alnorch E, *J. Pharm. Pharmacol.* 1998; **50**: 237-241

[44] Ravikanth V, Reddy VLN, Rao TP, Diwan PV, Ramakrishna S, Venkateswarlu Y, *Phytochemistry* 2002; **59**: 331-335

[45] Teuscher E, Lindequist U, *Biogene Gifte, Biologie – Chemie – Pharmakologie*, Stuttgart, Jena, New York: Gustav Fischer 1994. pp 136-147.

[46] Vogg G, Achatz S, Kettrup A, Sandermann H Jr., *J. Chromatogr. A* 1999; **855**: 563-573

[47] El-Mekkavy S, Meselhy MR, Nakamura N, Hattori M, Kawahata T, Otake T, *Phytochemistry* 2000; **53**: 457-464

[48] El-Mekkavy S, Meselhy MR, Nakamura N, Hattori M, Kawahata T, Otake T, *Chem. Pharm. Bull.* 1999; **47**: 1346-1347

[49] Gustafson KR, Cardellina JH, McMahon JB, Gulakowski RJ, Ishitoya J, Szallasi Z, Lewin NE, Blumberg PM, Weislow OS, Beutler JA, Buckheit RW Jr., Cragg GM, Cox PA, Bader JP, Boyd MR, *J. Med. Chem.* 1992; **35**: 1978-1986

[50] Fatope MO, Zeng L, Ohayaga JE, Shi G, McLaughlin L, *J. Med. Chem.* 1996; **39**: 1005-1008

[51] Marston A, Hecker E, *Planta Med.* 1983; **47**: 141-147

[52] Zani CL, Marston A, Hamburger M, Hostettmann K, *Phytochemistry* 1993; **34**: 89-95

[53] Wu TS, Lin YM, Haruna M, Pan DJ, Shingu T, Chen YP, Lee KH, *J. Nat. Prod.* 1991; **54**: 823-829

[54] Matsumoto TS, Cyong Jc, Yamada H, *Planta Med.* 1992; **58**: 255-258

[55] Zheng WF, Cui Z, Zhu Q, *Planta Med.* 1998; **64**: 754-756

[56] Kasai R, Lee KH, Huang HC, *Phytochemistry* 1981; **20**: 2592-2594

[57] Taninaka H, Takaishi Y, Honda G, Imakura Y, Sezik E, Yesilada E, *Phytochemistry* 1999; **52**: 1525-1529

[58] Brooks G, Evans AT, Aitken A, Evans FJ, Rizk AM, Hammouda FM, El-Missiry MM, Ismail SE, *Phytochemistry* 1990; **29**: 2235-2237

[59] Borris RP, Cordell GA, *J. Nat. Prod.* 1984; **47**: 270-278

[60] He W, Cik M, Puyvelde LV, Dun JV, Appendino G, Lesage A, Lindin IV, Leysen JE, Wouters W, Mathenge SG, Mudida FP, Kimpe ND, *Bioorgan. Med. Chem.* 2002; **10**: 3245-3255

[61] Abe F, Iwase Y, Yamauchi T, Kinjo K, Yaga S, *Phytochemistry* 1997; **44**: 643-647

[62] Adolf W, Sorg B, Hergenhahn M, Hecker E, *J. Nat. Prod.* 1982; **45**: 347-354

[63] Karalai C, Wiryachitra P, Sorg B, Hecker E, *Planta Med.* 1994; **60**: 566-568

[64] Tcinda AT, Tsopmo A, Tene M, Kamnaing P, Ngnokam D, Tane P, Ayafor JF, Connolly JD, Farrugia LJ, *Phytochemistry* 2003; **64**: 575-581

[65] Carney JR, Krenisky JM, Williamson RT, Luo J, Carlson TJ, Hsu VL, Moswa JL, *J. Nat. Prod.* 1999; **62**: 345-347

[66] Szallasi A, Fowler CJ, *Lancet Neurol.* 2002; **1**: 157-172

[67] Öksüz S, Gürek F, Gil RR, Pengsuparp T, Pezzuto JM, Cordell GA, *Phytochemistry* 1995; **38**: 1457-1462

[68] Zahid M, Husani SR, Abbas M, Pan Y, Jassbi AR, Asim M, Parvez M, Voelter W, Ahmad VU, *Helv. Chim. Acta* 2001; **84**: 1980-1988

[69] Ahmad VU, Hussain H, Jassbi AR, Hussain J, Bukhari IA, Yasin A, Aziz N, Choudhary MI, *J. Nat. Prod.* 2003; **66:** 1221-1224

[70] Ahmad VU, Jassbi AR, *J. Nat. Prod.* 1999; **62**: 1016-1018

[71] Öksüz S, Gürek F, Qiu SX, Cordell GA, *J. Nat. Prod.* 1998; **61**: 1198-1201

[72] Öksüz S, Gürek F, Yang SW, Lin LZ, Cordell GA, Pezzuto JM, Wagner H, Lotter H, *Tetrahedron* 1997; **53**: 3215-3222

[73] Ferreira MJU, Madureira AM, Ascenso JR, *Phytochemistry* 1998; **49**: 179-183

[74] Abdelgaleil SAM, Kassem SMI, Doe M, Baba M, Nakatani M, *Phytochemistry* 2001, **58**: 1135-1139

[75] Roengsumran S, Petsom A, Kuptiyanuwat N, Vilaivan T, Ngamrojnavanich N, Chaichantipyuth C, Phuthong S, *Phytochemistry* 2001; **56**: 103-107

[76] Konishi T, Yamazoe K, Konoshima T, Fujiwara Y, *Phytochemistry* 2003, **64**: 835-840

[77] Vigor C, Fabre N, Fourasté I, Moulis C, *Phytochemistry* 2001, **57**: 1209-1212

[78] Ngadjui BT, Abegaz BM, Keumedjio F, Folefoc GN, Kapche GWF, *Phytochemistry* 2002; **60**: 345-349

[79] Che CT, Zhou TX, Ma QG, Quin GW, Williams ID, Wu HM, Shi ZS, *Phytochemistry* 1999; **52**: 117-121

[80] Morgenstern T, Bittner M, Silva M, Aqueveque P, Jakupovic J, *Phytochemistry* 1996; **41**: 1149-1153

[81] Anjaneyulu ASR, Rao VL, Sreedhar K, *J. Nat. Prod.* 2002; **65:** 382-385

[82] Konishi T, Konoshima T, Fujiwara Y, Kiyosawa S, *J. Nat. Prod.* 2000; **63**: 344-346

[83] Ng AS, *Phytochemistry* 1990; **29**: 662-664

[84] Lal AR, Cambie RC, Rutledge PS, Woodgate PD, Rickard CEF, Clark GR, *Tetrahedron Lett.* 1989; **30**: 3205-3208

[85] Sahai R, Rastogi RP, Jakupovic J, Bohlmann F, *Phytochemistry* 1981; **20**: 1665-1667

[86] Valente C, Ferreira MJU, Abreu PM, Pedro M, Cerqueira F, Nascimento MSJ, *Planta Med.* 2003; **69**: 361-366

[87] Seip EH, Hecker E, *Phytochemistry*, 1984; **23**: 1689-1694

[88] Shizuri Y, Kosemura S, Ohtsuka J, Terada Y, Yamamura S, Ohba S, Ito M, Saito Y, *Tetrahedron Lett.* 1984; **25**: 1155-1158

[89] Yamamura S, Shizuri Y, Kosemura S, Ohtsuka J, Tayama T, Ohba S, Ito M, Saito Y, Terada Y, *Phytochemistry* 1989; **28**: 3421-3436

[90] Jakupovic J, Morgenstern T, Marco JA, Brendsohn W, *Phytochemistry* 1998; **47**: 1611-1619

[91] Li P, Feng ZX, Ye D, Huan W, Gang WD, Dong LX, *Helv. Chim. Acta* 2003; **86**: 2525-2532

[92] Fakunle CO, Connolly JD, Rycroft DS, *Fitoterapia* 1992; **63**: 329-332

[93] Uemura D, Katayama C, Uno E, Sasaki K, Hirata Y, Chen YP, Hsu HY, *Tetrahedron Lett.* 1975; **21**: 1703-1706

[94] Hohmann J, Evanics F, Dombi G, Szabó P, *Tetrahedron Lett.* 2001; **42**: 6581-6584

[95] Jakupovic J, Jeske F, Morgenstern T, Tsichritzis F, Marco A, Brendsohn W, *Phytochemistry* 1998; **47**: 1583-1600

[96] Marco JA, Sanz-Cervera JF, Yuste A, Jakupovic J, *J. Nat. Prod.* 1999; **62**: 110-113

[97] Corea G, Fattorusso E, Lanzotti V, Taglialatela-Scafati O, Appendino G, Ballero M, Simon PN, Dumontet C, Di Pietro A, *Bioorgan. Med. Chem.* 2003; **11**: 5221-5227

[98] Uemura D, Hirata Y, Chen YP, Hsu HY, *Tetrahedron Lett.* 1975; **21**: 1697-1700

[99] Hohmann J, Evanics F, Dombi G, Molnár J, Szabó P, *Tetrahedron* 2001; **57**: 211-215

[100] Liu LG, Meng JC, Wu SX, Li XY, Zhao XC, Tan RX, *Planta Med.* 2002; **68**: 244-248

[101] Kosemura S, Shizuri Y, Yamamura S, *Bull. Chem. Soc. Jpn.* 1985; **58**: 3112-3117

[102] Wang LY, Wang NL, Yao XS, Miyata S, Kitanaka S, *Chem. Pharm. Bull.* 2003; **51**: 935-941

[103] Marco JA, Sanz-Cervera JF, Yuste A, Jakupovic J, Lex J, *J. Org. Chem.* 1996; **61**: 1707-1709

[104] Marco JA, Sanz-Cervera JF, Yuste A, Jakupovic J, *Phytochemistry* 1997; **45**: 137-140

[105] Jakupovic J, Morgenstern T, Bittner M, Silva M, *Phytochemistry* 1998; **47**:1601-1609

[106] Liu LG, Tan RX, *J. Nat. Prod.* 2001; **64**: 1064-1068

[107] Marco JA, Sanz-Cervera J, Yuste A, Jakupovic J, Jeske F, *Phytochemistry* 1998; **47**: 1621-1630

[108] Wang LY, Wang NL, Yao XS, Miyata S, Kitanaka S, *J. Nat. Prod.* 2002; **65**: 1246-1251

[109] Ferreira AMVD, Carvalho LHM, Carvalho MJM, Sequeira AMS, *Phytochemistry* 2002; **61**: 373-377

[110] Uemura D, Hirata Y, *Tetrahedron Lett.* 1975; **21**: 1701-1702

[111] Appendino G, Jakupovic S, Tron JC, Jakupovic J, Milon V, Ballero M, *J. Nat. Prod.* 1998; **61**: 749-756

[112] Fischer NH, Olivier EJ, Fischer HD, The biogenesis and chemistry of sesquiterpene lactones. In: Herz W, Grisebach H, Kirby GW, eds. *Progress in the Chemistry of Organic Natural Products*, Vol 38, Wien, New York: Springer 1979. pp 59-60.

[113] Appendino G, Cravotto, Jarevång T, Sterner O, *Eur. J. Org. Chem.* 2000; **16**: 2933-2938

[114] Ulziykhutag N: *Latin-Mongolian-Russian Dictionary of Names of Taxonomy of the Vascular Plants of the MRP*. Ulaanbaatar: Academic Press 1983.

[115] Baikov KS, Euphorbiaceae. In: Peshkova GA ed. *Flora Sibiri*, Vol 10. Novosibirsk: Nauka Press 1996. pp 38-58.

[116] Borhidi A, *A zárvatermők fejlődéstörténeti rendszertana.* Budapest: Nemzeti Tankönyvkiadó 1995. pp 195, 230-234, 407-409.

[117] Soó R, *A magyar flóra és vegetáció rendszertani-növényföldrajzi kézikönyve*, Vol 2. Budapest: Akadémiai Kiadó 1966. p 598.

[118] Dános B, *Farmakobotanika, A gyógynövénytan alapjai (Kemotaxonómia).* Budapest: Argumentum 2002. p 210-248.

[119] Tutin TG, Heywood VH, Burges NA, Moore DM, Valentine DH, Walters SM, Webb DA, eds. *Flora Europea*, Vol 2. Cambridge: University Press 1968. pp 211-220.

[120] Simon T, *A magyarországi flóra határozója, Harasztok – virágos növények.* Budapest: Nemzeti Tankönyvkiadó 2000. pp 324-329.

[121] Shishkin BK, Bobrow EG, eds. *Flora SSSR*, Vol 14. Moscow, Leningrad: Akademia SSSR 1949. pp 342-343.

[122] Kinghorn AD, Evans FJ, *Planta Med.* 1975; **28**: 325-335

[123] Evans FJ, Kinghorn AD, *Bot. J. Linn. Soc.* 1977; **74**: 23-35

[124] Pohl R, Janistyn B, Nahrstedt A, *Planta Med.* 1975; **27**: 301-303

[125] Aseeva T, Blinova KF, Jakovlev GP, *Lekarstvennye Rasteniya Tibetskoi Meditsini.* Novosibirsk: Nauka Press 1985.

[126] Yamamura S, Kosemura S, Ohba S, Ito M, Saito Y, *Tetrahedron Lett.* 1981; **22**: 5315-5318

[127] Fakunle CO, Connolly JD, Rycroft DS, *J. Nat. Prod.* 1989; **52**: 279-283

[128] Betancur-Galvis L, Checa J, Marco JA, Estornell E, *Planta Med.* 2003; **69**, 177-178

[129] Appendino G, Spagliardi P, Ballero M, Seu G, *Fitoterapia* 2002; **73**: 576-582

[130] Corea G, Fattorusso E, Lanzotti V, Taglialatela-Scafati O, Appendino G, Ballero M, Simon PN, Dumontet C, Di Pietro A, *J. Med. Chem.* 2003; **46**: 3395-3402

[131] Hohmann J, Forgo P, Csupor D, Schlosser G, *Helv. Chim. Acta* 2003; **86**: 3386-3393

[132] Szabó D, Keyzer H, Kaiser HE, Molnár J, *Anticancer Res.* 2000; **20**: 4261-4274

[133] Sun J, He Z-G, Cheng G, Wang SJ, Hao X-H, Zou M-J, *Med. Sci. Monit.* 2004; **10**: 5-14

Acknowledgements

I first wish to thank Professor Imre Máthé for providing me with the opportunity to carry out my scientific work at the Department of Pharmacognosy. His human and mental support have greatly helped me to achieve my aims.

I would like to express my deepest gratitude to Professor Judit Hohmann, my supervisor, for her guidance, encouragement and management of my work. Her scientific enthusiasm has stimulated my interest in research tremendously. I will always value her exceptional determination, persistency and assiduity.

I am grateful to my brother, Tamás Rédei (Institute of Ecology and Botany of the Hungarian Academy of Sciences) and Dr. Tomur Zorig (Institute of Medicinal Research, Section of Pharmacy, Ulaanbaatar) for the collection of the plant material.

My special thanks go to Dr. Péter Forgó (Department of Organic Chemistry, University of Szeged) and Ferenc Evanics (Department of Pharmaceutical Analysis, University of Szeged) for the NMR measurements; to Dr. Tibor Bartók (Cereal Research Non-Profit Company) and Dr. Pál Szabó (Institute of Chemistry, Chemical Research Centre of the Hungarian Academy of Sciences) for the mass spectra; to Professor Alajos Kálmán and Dr. Gyula Argay (Institute of Chemistry, Chemical Research Centre of the Hungarian Academy of Sciences) for the X-ray data; and to Professor József Molnár and Dr. Ilona Mucsi (Department of Medical Microbiology, University of Szeged) for the anti-MDR, antiviral and cytotoxicity investigations.

My thanks are also due to all the staff members and laboratory personnel, especially Erzsébet Berta, who helped in any way during my research.

I am particularly grateful and indebted to my parents for giving me so much emotional support.

Appendix

The thesis is based on the following publications referred to in the text:

I Hohmann J, Rédei D, Evanics F, Kálmán A, Argay G, Bartók T: Serrulatin A and B, new diterpene polyesters from *Euphorbia serrulata*, *Tetrahedron* 2000; **56**: 3619-3623

II Mucsi I, Molnár J, Hohmann J, Rédei D: Cytotoxicities and anti-herpes simplex virus activities of diterpenes isolated from *Euphorbia* species, *Planta Med.* 2001; **67**: 672-674

III Hohmann J, Molnár J, Rédei D, Evanics F, Forgo P, Kálmán A, Argay G, Szabó P: Discovery and biological evaluation of a new family of potent modulators of multidrug resistance: Reversal of multidrug resistance of mouse lymphoma cells by new natural jatrophane diterpenoids isolated from *Euphorbia* species, *J. Med. Chem.* 2002; **45**: 2425-2431

IV Rédei D, Hohmann J, Evanics F, Forgo P, Szabó P, Máthé I: Isolation and structural characterization of new, highly functionalized diterpenes from *Euphorbia serrulata*, *Helv. Chim. Acta* 2003; **86**: 280-289

V Hohmann J, Rédei D, Forgo P, Molnár J, Dombi G, Zorig T: Jatrophane diterpenoids from *Euphorbia mongolica* as modulators of the multidrug resistance of L5128 mouse lymphoma cells, *J. Nat. Prod.* 2003; **66**: 976-979